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PROCESS DEVELOPMENT FOR PLUG FLOW ACID HYDROLYSIS AND CONVERSION OF LIGNOCELLULOSICS TO ETHANOL

PREPARED FOR:

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EXECUTIVE SUMMARY

A process was developed at the pilot plant scale for converting both pine and aspen wood to ethanol. The process consisted initially of hydrolyzing the hemicellulose fraction of the wood (prehydrolysis) in a Wenger single screw extruder at yields of up to 24.7% for pine and 22.9% for aspen, based on the C_5/C_6 sugars produced as a function of the wood feed. The extruder was found to be capable of processing sawdust wood feeds (on a continuous basis) of up to 68% D.S. and utilized SO_2 as the catalyst.

The extruded material was then passed through a washing system utilizing a plate and frame filter press. Mass balances performed around the filter revealed that approximately 95% of the sugars produced through the extruder could be recovered in a two stage washing process.

Delignification experiments were then performed to try to recover the lignin contained in the extruded wood before the hydrolysis stage. The results from the experiments performed primarily with NaOH, demonstrated that the lignin can only be partially removed from the wood (50% for aspen, 30% for pine) and that a high percent of the original cellulose material is lost (>50%) during processing.

The washed extruded material was then passed through the SLR plug flow reactor. Feeds of up to 17% D.S. were able to be processed through the reactor. The maximum yields obtained were 21.9% and 18.7% for aspen and pine respectively, based on the amount of glucose produced as a function of the original wood feed.

The hydrolyzed material was diluted and passed through either a decanter or centrifuge to recover the sugars produced. Mass balances performed revealed that approximately 75% of the sugars produced in the hydrolysis stage could be recovered in a single stage washing process, while approximately 95% of the sugars produced could be recovered in a two stage washing process.

The sugar streams recovered from both the prehydrolysis and hydrolysis processes were fermented separately and together, with primarily Pichia stipitis, in fermentation vessels of up to 28-L. Yields ranging between 0.43 and 0.49 were obtained from fermentations utilizing sugar concentrations of up to 116-g/L. The fermentation runs lasted for between 12 and 68 hours.

An overall mass balance based on the results obtained in the pilot plant was developed for a proposed 20,000,000-L/yr wood to ethanol plant, integrated with a pulp and paper mill, located in Northern Ontario. The ethanol plant would utilize primarily the pine waste wood from the pulp plant which is presently being used to generate steam and electricity. From the mass balance an overall process yield of 217-L/ODMT was obtained. This yield is lower than was estimated in the previous study (approximately 314-L/yr) due primarily to the consideration of losses associated with the two washing stages, a lower yield obtained through the use of the Wenger extruder compared to that obtained from the SLR plug flow reactor for prehydrolysis and the fact that recycling of the hydrolyzed material was found to be uneconomical. It is important to note that although the yields obtained with the Wenger extruder are lower than those obtained with the SLR plug flow reactor for prehydrolysis, the ability of the Wenger extruder to process feeds of a high dry solids content (>50%) makes it a more economical reactor to utilize than the plug flow reactor in which the dry solids content of the feed is limited to approximately 5% for prehydrolysis.

It was estimated that the ethanol plant would have a fixed capital cost of $18,500,000. Based on a feedstock cost of $46.20/ODMT, the fuel value equivalent of the wood, and a selling price for ethanol of $0.40/L the plant would have a negative IRR if the lignin rich residue (>60% lignin) produced from the plug flow reactor had a value equivalent to its fuel value. It was determined that an IRR of 22% could be obtained if the residue had a net value of $0.10/lbs. Research is presently being conducted at Bio-Hol to determine the value of the residue material and methods to process it to obtain the maximum value from both the cellulose and lignin fractions.

RÉSUMÉ

Il s'agit d'un procédé mis au point dans une usine pilote permettant de convertir le bois de pin et de tremble en éthanol. Le procédé consistait à hydrolyser de l'hémicellulose de bois (préhydrolyse) à l'aide d'une extrudeuse Wenger à vis unique capable de convertir le bois en sucres composés de 5 et 6 carbones avec un rendement de 24.7% pour le pin et de 22.9% pour le tremble. L'extrudeuse, qui utilisait du SO_2 comme catalyseur, s'est révélée capable de traiter de la sciure de bois, alimentée sur une base continue, contenant jusqu'à 68% de matières sèches.

Le matériel extrudé fut ensuite soumis à un lavage à l'aide d'une filtre presse à plateau. Les équilibres de masse réalisés autour du filtre indiquent qu'approximativement 95% des sucres produits par extrusion sont récupérables par un procédé de lavage à deux étapes.

On a ensuite essayé de récupérer la lignine contenue dans le bois extrudé avant l'étape d'hydrolyse. Les résultats de ces essais effectués principalement avec du NaOH démontrent que la lignine ne peut être que partiellement séparée du bois (50% dans le cas du tremble et 30% dans celui du pin) et qu'un gros pourcentage de la cellulose de départ est perdue (>50%) lors du traitement.

Une fois extrudé et lavé, le matériel fut soumis à l'action d'un réacteur SLR d'écoulement à bouchon. Il a été possible d'alimenter le réacteur avec du matériel contenant jusqu'à 17% de matières sèches. Le rendement maximum obtenu a été de 21.9% pour le tremble et de 18.7% pour le pin, sur la base du montant de glucose produit en fonction de la quantité de bois fournie.

Le matériel hydrolysé fut dilué et soumis à l'action d'une décanteuse ou d'une centrifugeuse afin de récupérer les sucres produits. L'équilibre statique révèle qu'approximativement 75% des sucres produits à l'étape de l'hydrolyse sont récupérables par un procédé de lavage d'une seule étape, mais qu'en deux étapes il est possible de récupérer 95% des sucres.

Les sucres recueillis à l'aide des procédés de préhydrolyse furent fermentés séparément et ensemble, à l'aide principalement de la bactérie *Pichia stipitis*, dans des contenants d'une capacité de 28 litres et moins. Un rendement variant de 0.43 et de 0.49 fut obtenu à partir des fermentations utilisant des concentrations de sucres allant jusqu'à 116 g/L. Les fermentations durèrent de 12 à 68 heures.

Un bilan massique des résultats obtenus à l'usine pilote fut établi en prévision de la mise sur pied d'une usine de production d'éthanol d'une capacité de 20,000,000 L/année, qui serait annexée à un moulin de pâte et papier, située dans le nord de L'Ontario. L'usine d'éthanol serait principalement alimentée des

déchets de pins en provenance du moulin de pâte, qui servent présentement à produire de la vapeur et de l'électricité. Le bilan massique a permis de projeter un rendement global de 217 L/tonne métrique de matière séchée au four. Ce rendement est moindre que celui que l'on avait projeté lors d'une étude précédente (approximativement 314 L). Cet écart s'explique principalement par les pertes qui surviennent lors des deux étapes de lavage, par le faible rendement obtenus lors de l'utilisation de l'extrudeuse Wenger comparativement au rendement obtenu lors de l'utilisation du réacteur SLR pour la préhydrolyse et par le fait que le recyclage du matériel hydrolysé ne s'avère pas constituer une étape rentable. Il est important de noter que même si le rendement obtenu avec l'extrudeuse Wenger est plus faible que celui obtenu avec le réacteur SLR utilisé pour la préhydrolyse, l'extrudeuse Wenger pouvait traiter des déchets de bois contenant une grande quantité de matières sèches (>50%) ce qui la rend plus économique que le réacteur à bouchon dans lequel l'utilisation pour l'hydrolyse des matières sèches est limitée à approximativement 5%.

Il est estimé que l'usine de méthanol représenterait un coût fixe d'investissement de 18,500,500 $. Pour un coût d'approvisionnement de 46.20 $/tonne métrique de matière séchée au four et un prix de vente de l'éthanol de 0.40 $/L, l'usine représenterait un taux de rendement effectif négatif si les déchets à haute teneur de lignine (>60%) produits à partir du réacteur à bouchon avaient une valeur équivalente à leur valeur comme carburant. Il fut déterminé qu'il serait possible d'obtenir un taux de rendement effectif de 22% si la valeur des déchets était de 0.10 $/lb. Les recherches se poursuivent présentement à Bio-Hol dans le but de déterminer la valeur du matériel résiduel ainsi que les méthodes aptes à le traiter qui permettraient de tirer le meilleur rendement possible de la lignine et de la cellulose.

PREFACE

This document constitutes the final report for work commenced February 1987 over a 24 month period under a co-operative funding agreement between Bio-Hol Developments, a joint venture between St. Lawrence Reactors and George Weston Limited, the Ontario Ministry of Energy, and Energy, Mines and Resources entitled, "Process Development for Plug Flow Acid Hydrolysis and Conversion of Lignocellulosics to Ethanol".

The fermentation component of this study was subcontracted to the University of Toronto under the supervision of Professor Morris Wayman of the Department of Chemical Engineering and Applied Chemistry.

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GLOSSARY OF TERMS

%Dry Solids (%D.S.) - The percentage of both soluble and insoluble material that remains after drying a known weight of material.

Prehydrolysis - The acid catalyzed reaction in which the hemicellulose fraction of the wood is converted into its constituent sugars.

Prehydrolysate - The product obtained from prehydrolysis. This term can refer to either the solid or liquid fraction of the product.

Hydrolysis - The acid catalyzed reaction in which the cellulose fraction of the wood is converted into its constituent sugar, glucose.

Hydrolysate - The material obtained from hydrolysis.

5-HMF - Hydroxymethylfurfural, a decomposition product of glucose that is formed during cellulose hydrolysis.

ODMT - Oven dried metric tonne.

IRR - Internal rate of return.

1.0 INTRODUCTION

The work performed under this research agreement was a continuation of the work previously undertaken by St. Lawrence Reactors and the University of Toronto under a funding agreement with the Ontario Ministry of Energy entitled, "Research into Cost Effective Fuel Ethanol Processes from Indigenous Raw Material Sources". In that project a process, incorporating the SLR plug flow reactor for both hemicellulose and cellulose hydrolysis, for converting various types of cellulosic materials to ethanol was investigated. It was determined that although the plug flow reactor was able to hydrolyze the hemicellulose fraction of wood at a high yield (approximately 90%), the reactor was only capable of processing wood slurries of 5% D.S., which was deemed unfeasible for a commercial process.

The objective of this project was to investigate a wood to ethanol process incorporating a Wenger single screw extruder for hemicellulose hydrolysis (prehydrolysis) and the SLR plug flow reactor for cellulose hydrolysis. A Wenger single screw extruder was chosen as a substitute for the SLR plug flow reactor for prehydrolysis, due to its ability to handle wood slurries of a high dry solids content (>50%). Through experimentation an overall process scheme would be developed and optimized and the economics for a semi-commercial plant would be analyzed.

2.0 COMPANY PROFILE

Bio-Hol Developments is a joint venture between St. Lawrence Reactors and George Weston Limited. St. Lawrence Reactors is a developer and supplier of process technology for the conversion of biomass into value-added products. The company has developed and patented a plug flow reactor which is used for the production of maltodextrins and glucose syrups for the corn wet-milling industry and also the acid catalyzed conversion of a variety of feedstocks. St. Lawrence Starch Limited the majority shareholder of St. Lawrence Reactors operates both a fuel and potable ethanol plant. Through this association St. Lawrence Reactors has gained considerable expertise into the commercial production of ethanol.

George Weston Limited is a broadly based company conducting food processing, food distribution and resource operations. Weston, through its corporate research facility Weston Research Centre, has been involved in the field of food research and development. In 1983, the Bio-Hol Developments pilot plant was established for the purpose of developing and demonstrating a process for the conversion of wood to fuel ethanol based on Zymomonas fermentation technology. In 1986 St. Lawrence Reactors became partners with George Weston in Bio-Hol Developments.

The fermentation work covered in this project was performed by Professor Morris Wayman of the University of Toronto. Professor Wayman is an authority on the production of fermentable sugars from cellulosics and has made advancements into solving the problem of fermenting pentose sugars.

3.0 EQUIPMENT

3.1 Batch Reactor

A batch reactor was constructed for this project to permit the investigation of both prehydrolysis and delignification. The reactor has a capacity of approximately 35-Kg and was designed to operate at a maximum temperature of 200°C and a maximum pressure of 600-psig. The reactor contains a mixer and can be heated both directly through the injection of steam and indirectly through steam heated coils.

3.2 Wenger Single Screw Extruder

A Wenger extruder was chosen as the apparatus to be used for prehydrolysis. This extruder had been previously used successfully for the H_2SO_4 catalyzed hydrolysis of both aspen and pine. In previous work the extruder was found capable of processing wood sawdust of a high dry solids content (approximately 50%) and operating at pressures up to 1500-psig and temperatures up to 230°C.

The system used consisted of: a paddle mixer in which the moisture of the wood feed was adjusted; a vibrator and cyclone vacuum system to transfer the feed to the live bin of the extruder; a screw feeder to the extruder including an acid injection port. The temperature and pressure of the extruder were monitored by a strip chart recorder during each run. Detailed information concerning the extruder system is found in Appendix I.

3.3 SLR Plug Flow Reactor

The reactor system used consisted of two sections: two low pressure shells which were used to heat up the feed material to approximately 167°C; one high pressure shell in which temperatures of up to 230°C were attained. The shells each contained three sets of 1-in tubing in which the material travelled. The tubing was constructed of a special ferritic steel and was indirectly heated with steam. A Robbins and Myers 9P4 progressive cavity Moyno pump was used to feed the reactor. A 0.15-in orifice was used to maintain the back pressure within the reactor.

The reactor system was used both for acid catalyzed hydrolysis and for delignification experiments.

3.4 Separation Equipment

A Perrin plate and frame filter press was used for washing the extruded prehydrolyzed wood material to recover the sugars produced.

A Westfalia CA 220 decanter/clarifier and a Westfalia SB 14 de-sludger/clarifier were both used for the solid/liquid separation of the plug flow hydrolyzed material.

3.5 Sugar Concentration Determination

Sugar concentrations were determined through a High Performance Liquid Chromatography (HPLC) which consisted of a Bio-Rad HPX 87H column, a Waters R-401 Differential Refractometer, a Beckman 110 B Solvent Delivery Module and a Shimadzu C-R3A Chromatopac Integrator. The column temperature was 40°C using a mobile phase of 0.01-N H_2SO_4 and a flowrate of 0.60-mL/min. Quantitation was performed using the external standard method and multi-level calibration.

4.0 EXPERIMENTAL PROCEDURES

4.1 Analytical Methods

The analyses performed in this project consisted of determining the: dry solids content; soluble/insoluble fractions; lignin, cellulose, hemicellulose and extractives content; identification of solubles. The methods utilized are outlined in detail in Appendix II.

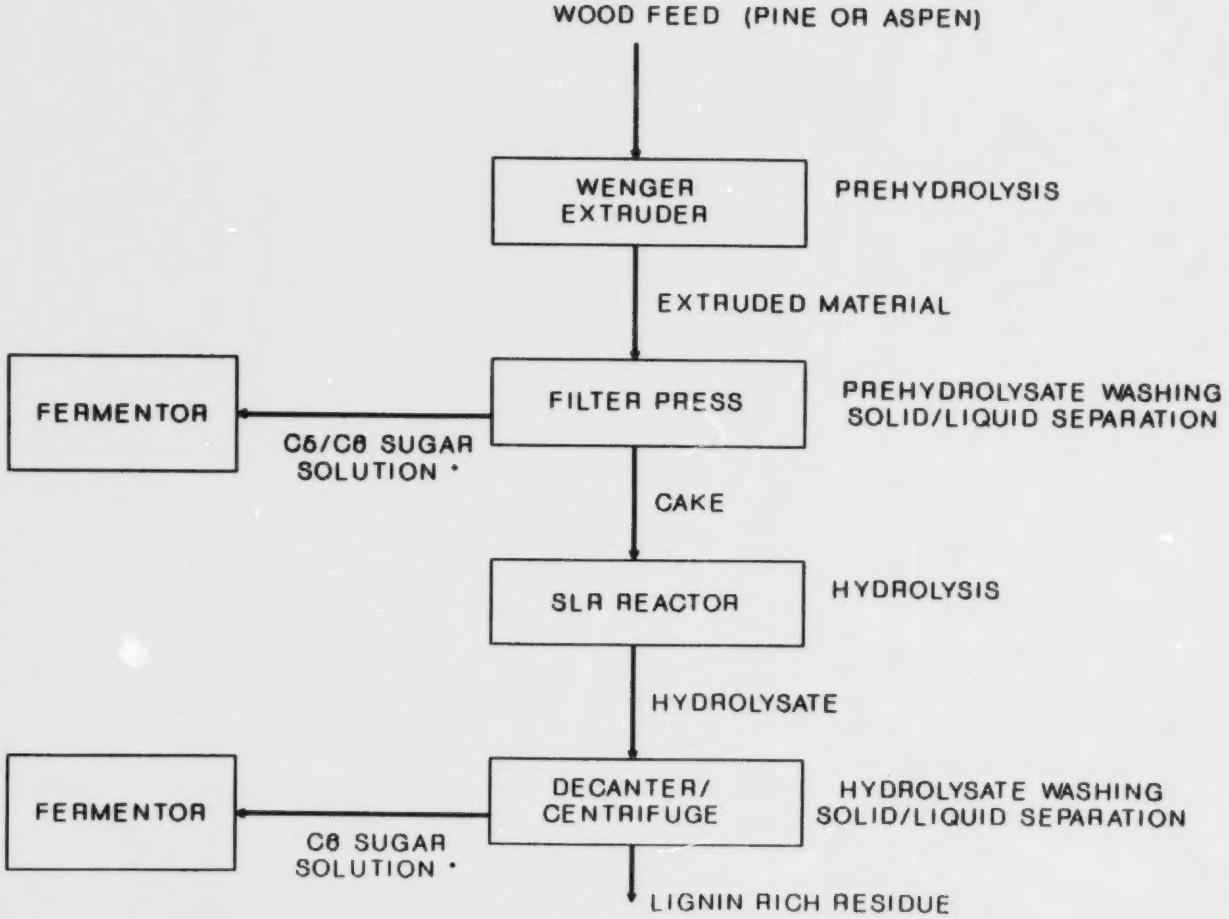
4.2 Experimentation

The pilot plant flowsheet is shown in Figure no. 1. Wood slurries of both pine and aspen ranging in dry solids content of between 55 and 60%, and 55 and 70% respectively, were prepared in the paddle mixer. The wood slurry was fed into the extruder at an acid loading ranging from 1.0 to 3.0% SO_2/dry wood feed. During the course of each experiment the temperature and pressure along the barrel of the extruder were monitored. The extruder was operated at temperatures and pressures of up to 180°C and 1200-psig respectively at the outlet. The product was collected at timed intervals which permitted the mass flowrate of the product to be determined.

The extruded wood product was slurried up and passed into the filter press. The cake obtained was then processed through the plug flow reactor. The feed material to the reactor had a dry solids content of approximately 17%. The orifice and coil length utilized throughout the experimentation were 0.15-in and 120-ft respectively. The acid strength (HCl/(HCl + Water)) was maintained at 0.3%, which corresponded to a pH of 1.5 and an acid/wood of 1.5%. The residence time in the reactor was varied between 1.3 and 2.5 minutes and the temperature ranged from 215°C to 226°C. The temperature of the reactor was varied by adjusting the

FIGURE NO. 1

PILOT PLANT FLOWSHEET



* FERMENTED TOGETHER AND SEPARATELY

pressure of the boiler and the residence time in the reactor was controlled through the speed of the pump. The product obtained from the reactor was collected at timed intervals.

The hydrolyzed material was diluted with water and passed through the clarifier/decanter and or the desludger/clarifier to recover the sugar solution produced. The sugar solution was then concentrated for fermentation.

The prehydrolysate, hydrolysate and combined prehydrolysate and hydrolysate streams were fermented at the University of Toronto using shake flasks and a 4-L fermentor and at Bio-Hol in a 28-L fermentor. The fermentations were performed utilizing *Pichia stipitis*.

5.0 RESULTS AND DISCUSSION

5.1 Feedstock Analysis

The feedstock analysis was obtained from the Weston Research Centre (WRC). The analyses of both aspen and pine are listed in Table no.1. The analytical procedures used are outlined in Appendix II. These analyses were used throughout the report to determine sugar yields based on the individual components of the wood.

TABLE NO. 1

FEEDSTOCK ANALYSIS

	PINE	ASPEN
CELLULOSE	44.8%	50.6%
HEMICELLULOSE	19.0%	18.1%
LIGNIN	31.6%	24.1%
EXTRACTIVES	7.3%	4.9%

5.2 Prehydrolysis

5.2.1 Batch Reactor

A series of prehydrolysis runs (See Tables Nos. 2&3) were performed in the batch reactor. Initially the experiments were run (PDB1-6) utilizing a dilute slurry feed of pine (12-15.0%DS) and an acid strength (HCl) ranging between 0.3 and 0.4 %. Two experiments each were performed at 5,10 and 15 minutes. The maximum yield of 15.2% xylose, based on the dry wood feed was obtained from the experiment utilizing a 15 minute reaction time.

A series of runs (PB1-7, AB1-3) using a more concentrated feed (34.8-35.0 %D.S.) were then performed with both aspen and pine. The acid strength (HCl) used was 0.4% for all except one experiment (PB7), the reaction temperature ranged between 162°C and 167°C and the reaction time was varied between 5 and 20 minutes. The yields (% xylose/wood feed) obtained for both aspen and pine were comparable for the runs utilizing a reaction time of 5 minutes (pine:12.2 vs aspen:12.4) and 7.5 minutes (pine:12.8 vs aspen:13.8). In the runs with a 10 minute reaction time (PB1-2,AB3), there was a significant difference between the yields obtained, 15.7 (pine) vs 13.0 (aspen). The major difference in the products produced from the two woods was the amount of acetic acid produced. The acetic acid/wood (%) ranged between 1.0 and 1.2 for pine and ranged between 3.5 and 3.7 for the aspen.

TABLE NO. 2

BATCH HCl CATALYZED PREHYDROLYSIS RUNS

RUN NO.	REACTOR CHARGE (LBS)	DILUTION STEAM (LBS)	INITIAL DS (%)	FINAL DS (%)	FINAL HCl STRENGTH (%)	REACTION TEMP (DEG C)	REACTION TIME (1) (MIN)	XYLOSE/HC YIELD (2) (%)	XYLOSE/WOOD YIELD (%)	GLUCOSE/WOOD YIELD (%)	ACETIC ACID /WOOD (%)
PDB1	70.0	22.3	15.0	11.3	0.4	162.0	7/15/8	70.4	15.2	3.6	0.0
PDB2	70.0	28.8	15.0	9.0	0.3	163.0	8/15/15	67.6	14.6	3.8	0.0
PDB3	70.0	23.5	15.0	9.8	0.4	160.0	7/10/11	56.1	12.1	2.8	0.0
PDB4	70.0	26.8	15.0	9.6	0.3	158.0	8.5/10/9	62.5	13.5	2.5	0.0
PDB5	70.0	22.3	12.0	8.7	0.4	162.0	7/5/4	59.3	12.8	2.6	0.0
PDB6	70.0	25.8	12.0	8.8	0.4	161.0	8/5/5	54.3	11.7	2.5	0.0
PB1	33.0	25.8	34.8	18.1	0.4	164.0	4/10/5	78.3	16.9	4.6	1.1
PB2	33.0	18.8	34.8	20.9	0.4	164.0	6.5/10/5.5	66.7	14.4	3.4	1.1
PB3	33.0	22.5	34.8	19.5	0.4	162.0	6/20/7	64.4	13.9	4.3	1.2
PB4	33.0	20.3	34.8	19.9	0.4	164.0	6/15/7.5	60.7	13.1	4.0	1.2
PB5	33.0	18.3	34.8	21.7	0.4	167.0	6/7.5/5	57.4	12.4	3.1	1.0
PB6	33.0	19.5	34.8	20.6	0.4	166.0	7/7.5/7	61.2	13.2	3.4	1.1
PB7	33.0	15.8	34.8	24.4	0.5	166.0	6/5/5	56.5	12.2	2.8	1.0
AB1	40.0	17.5	35.0	21.3	0.4	166.0	7/5/4.5	60.3	12.4	1.6	3.5
AB2	40.0	17.5	35.0	21.1	0.4	167.0	7/7.5/5	67.1	13.8	2.8	3.7
AB3	40.0	21.3	35.0	22.2	0.4	166.0	7.5/10/5	63.2	13.0	2.0	3.5

Note: 1. The first number refers to the heat up time, the second number is the reaction time and the third number is the cool down time.

2. HC = hemicellulose in feed material

TABLE NO. 3

BATCH SO2 CATALYZED PREHYDROLYSIS RUNS

WOOD	PINE	ASPEN
FINAL SO2 STRENGTH (%)	1.9	1.8
SO2/WOOD (%)	5.9	5.9
REACTION TIME (MIN)	5.0	5.0
REACTION TEMP (DEG C)	168.0	168.0
SOLUBLES PRODUCED (%)	20.9	27.8
XYLOSE/HC YIELD (%)	42.2	66.1
XYLOSE/WOOD YIELD (%)	9.1	13.6
GLUCOSE/WOOD YIELD (%)	2.1	1.5
ACETIC ACID/WOOD (%)	0.7	2.8

The final series of runs performed in the batch reactor utilized SO_2 as the catalyst (Table no.3). These experiments were performed to obtain an indication of the levels of SO_2 to be used in the Wenger extruder. One run was performed with both aspen and pine. The SO_2/wood (%) used was 5.9, the reaction time was 5 minutes and the reaction temperature was 168°C for both experiments. The yields obtained (xylose/wood: 9.1% pine; 13.6% aspen) indicated that SO_2 can be used as a catalyst for the prehydrolysis of both pine and aspen.

5.2.2 Wenger Extruder

The results from the prehydrolysis runs performed with the Wenger extruder with both pine and aspen are shown in Table no.4. The data from the table comes from the flowsheets presented in Appendix IV. Initially the extruder was operated with a low flowrate (2.4-4.2 lbs/min). It was observed during these runs that the outlet flow was surging and that the product was inconsistent. The extruder was then shipped back to Wenger Manufacturing in order that the appropriate modification could be made to allow for a higher flowrate and smoother operation. The modifications made to the extruder system are shown in Figure no.2.

The modifications made to the extruder allowed for the feed rate to be increased by up to 207% and 263% for pine and aspen respectively. A smoother product flowrate and a consistent product were also obtained.

TABLE NO 4A

WENGER PINE PREHYDROLYSIS

RUN	FEED					REACTION CONDITIONS							
	WOOD (LBS/MIN)	D.S. (%)	SO2 FEED (LBS/MIN)	SO2 FEED (%)	SO2/WOOD (%)	T1 (DEG C)	T2 (DEG C)	T3 (DEG C)	T4 (DEG C)	T5 (DEG C)	P1 (PSIG)	P2 (PSIG)	P3 (PSIG)
WP-1	4.2	55.2	0.8	5.2	1.8	174.4	189.4	200.6	203.3	173.3	N/A	300.0	400.0
WP-2	5.2	55.0	0.9	3.6	1.1	168.3	176.7	196.1	207.2	175.0	250.0	350.0	450.0
WP-3	8.7	59.0	1.3	5.3	1.4	170.0	182.2	203.3	206.7	170.6	350.0	350.0	725.0

RUN	PRODUCT				% PRODUCT/DRY WOOD FEED					% CONVERSIONS	
	D.S. (%)	SOLUBLES (%)	CELLULOSE (%)	LIGNIN (%)	XYLOSE/ MANNOSE	GLUCOSE	ARABINOSE	ACETIC ACID	LACTIC ACID	XYLOSE/ HC (1)	CELLULOSE (2)
WP-1	57.5	32.0	38.5	53.6	15.6	5.4	1.3	-	1.6	72.3	39.2
WP-2	60.2	34.7	50.9	44.8	17.3	5.8	1.6	1.2	1.3	80.2	22.7
WP-3	63.8	33.1	51.4	43.7	17.6	4.0	1.3	1.3	-	81.4	20.1

TABLE NO 4B

WENGER ASPEN PREHYDROLYSIS

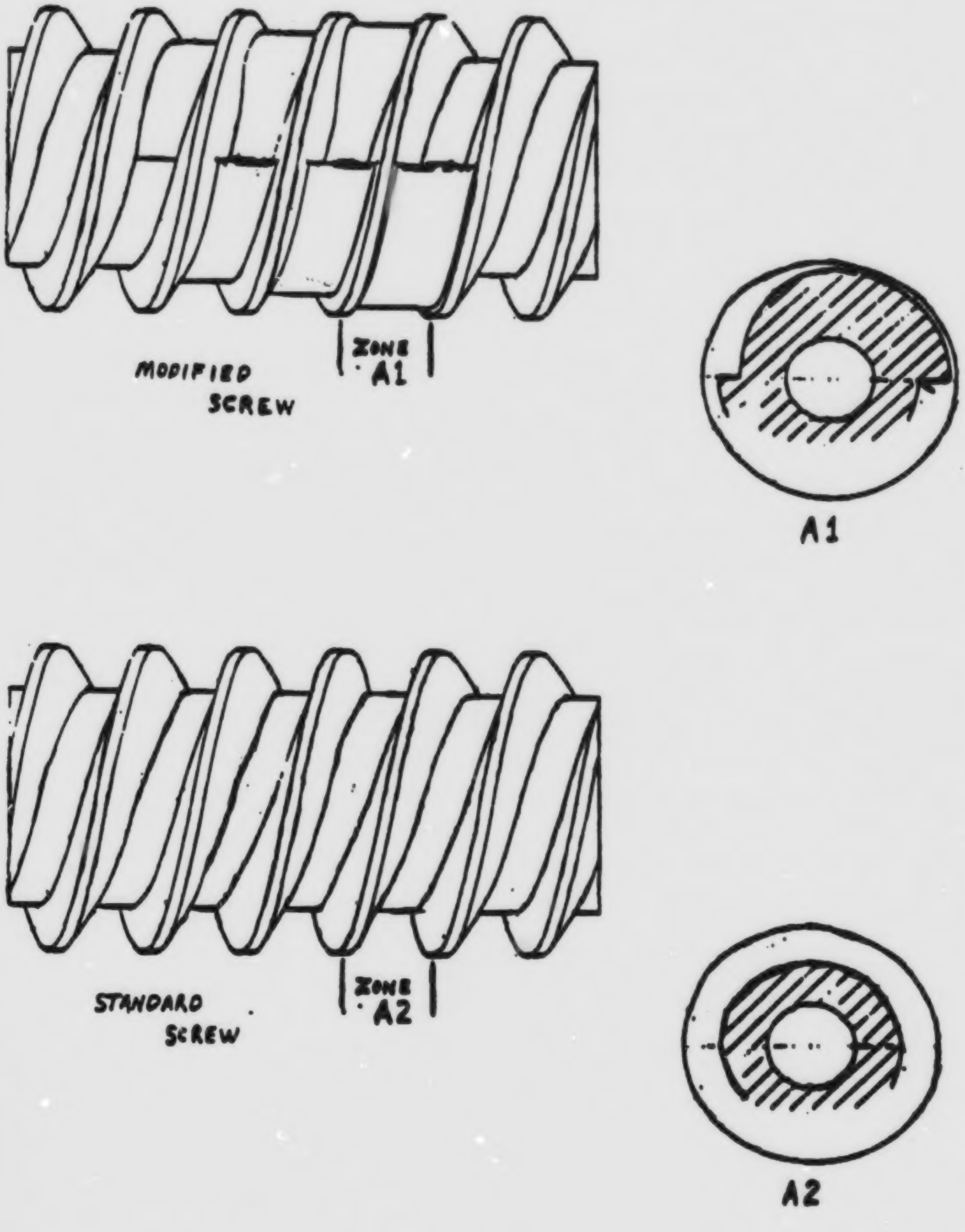
RUN	FEED					REACTION CONDITIONS							
	WOOD (LBS/MIN)	D.S. (%)	SO2 FEED (LBS/MIN)	SO2 FEED (LBS/MIN)	SO2/WOOD (LBS/MIN)	T1 (DEG C)	T2 (DEG C)	T3 (DEG C)	T4 (DEG C)	T5 (DEG C)	P1 (PSIG)	P2 (PSIG)	P3 (PSIG)
WA-1	2.4	68.1	1.3	3.6	2.8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
WA-2	4.0	56.6	1.3	2.4	1.4	140.6	196.1	198.9	193.3	167.2	N/A	N/A	N/A
WA-3	3.8	58.7	1.3	2.9	1.6	132.2	193.3	193.3	196.1	171.1	100.0	300.0	750.0
WA-4	6.0	67.2	1.2	4.9	1.5	204.4	208.9	204.4	206.1	175.6	150.0	150.0	250.0
WA-5	6.3	64.8	1.1	5.8	1.5	189.4	206.1	206.1	208.9	164.4	150.0	150.0	350.0

RUN	PRODUCT				% PRODUCT/DRY WOOD FEED					% CONVERSIONS	
	D.S. (%)	SOLUBLES (%)	CELLULOSE (%)	LIGNIN (%)	XYLOSE/ MANNOSE	GLUCOSE	ARABINOSE	ACETIC ACID	LACTIC ACID	XYLOSE/ HC (1)	CELLULOSE (2)
WA-1	54.9	38.8	66.1	31.4	14.5	4.4	-	1.8	-	70.4	
WA-2	52.1	37.0	51.3	39.4	15.8	3.8	-	3.3	-	76.8	33.2
WA-3	54.9	32.5	50.6	24.1	11.9	2.3	0.6	2.6	-	66.0	16.0
WA-4	67.2	33.8	61.9	30.5	18.8	3.7	0.4	3.7	-	80.7	15.6
WA-5	69.2	34.2	67.6	31.9	14.1	3.9	0.3	2.7	0.8	68.5	8.5

NOTE:
1. HC = Hemicellulose; Conversion is based on the amount of hemicellulose sugars produced as a function of the hemicellulose contained in the feed material
2. (cellulose in feed - cellulose in product)/cellulose in feed

FIGURE NO. 2

MODIFIED EXTRUDER SCREW



Drawing by Wenger Manufacturing

It was observed during the course of experimentation that the operation of the extruder was smoother when pine was used as the feed material. In addition, it was noted during the cleaning of the extruder, which took place after each run, that the aspen feed left a solid residue along the cone and the poppit area of the extruder.

The yields (% xylose/dry wood feed) ranged between 11.9 (WA-3) and 18.8 (WA-4), and 15.6 (WP-1)and 17.6 (WP-3) for aspen and pine respectively. The maximum % xylose/HC yield was 80.7% and 81.4% for aspen and pine respectively. The maximum yields from pine were obtained from the runs in which the outlet temperature was 170.6^{o}C and the % SO_2/wood was 1.4. The maximum yields from aspen were obtained from the run in which the outlet temperature was 175.6^{o}C and the %SO_2/wood was 1.5. The mass balances of each run are contained in Appendix III.

In the previous work performed utilizing the SLR reactor for prehydrolysis (1), the maximum yields (%xylose/wood) obtained were 20.0 and 19.9 for aspen and pine respectively. In comparing the maximum yields obtained from the SLR reactor and the Wenger extruder (pine:18.8 and aspen:17.6), it can be concluded that the maximum yields achieved with the SLR reactor were 6.4% (aspen) and 13.1% (pine) greater than those achieved with the extruder.

5.3 Washing of Extruded Material

The washing of the prehydrolyzed material was performed with a Perrin plate and frame filter press. To determine the washing efficiency of the material, mass balances were performed for various washes. The mass balances obtained are listed in Table no. 5.

A comparison can be made of the washing characteristics of extruded pine and aspen through washes no. WSPP-1 (pine) and WSPA-1 (aspen). In these washes the extruded material was passed through the filter press twice, diluted to between 18.0 and 20% D.S. for each pass. Through this series of washes an overall washing efficiency of 94.0% was obtained for the pine compared to 86.4% for the aspen. Wash no. WSPA-3 demonstrates that the aspen material must be diluted approximately twice as much as the pine material in the second wash step to accomplish roughly the same washing efficiency .

Wash no. WSPA-2 was performed to compare the washing efficiency between a two wash system (WSPA-1) and a one wash system, using equivalent amounts of water. The results obtained demonstrate that the washing efficiency of the two wash system was approximately 5.8% greater than the one wash system.

TABLE NO.5

WASHING OF EXTRUDED MATERIAL : PLATE AND FRAME FILTER PRESS

WASH NO	MATERIAL	PASS NO	D.S. FEED	%SOLUBLES FEED	%D.S. CAKE	%SOLUBLES CAKE	%SOLUBLES REMOVED	% SOLUBLES REMOVED OVERALL
WSPP-1	PINE:WP-3	1	18.5	34.0	40.2	11.7	73.7	
	PINE:WP-3	2	18.9	11.7	41.1	3.0	75.4	94.0
WSPA-1	ASPEN:WA-4	1	18.1	42.7	42.5	12.8	70.0	
	ASPEN:WA-4	2	20.0	12.8	37.4	5.8	54.7	86.4
WSPA-2	ASPEN:WA-5	1	11.8	34.9	43.7	9.0	81.4	81.4
WSPA-3	ASPEN:WA-5	1	18.2	34.3	43.8	13.4	71.9	
	ASPEN:WA-5	2	11.0	13.4	37.0	3.8	74.3	92.8

5.4 Delignification of Extruded Material

Delignification experiments were performed on the extruded material with both NaOH and acetone. The experiments performed with NaOH were run in the autoclave, batch reactor and plug flow reactor, while those performed with acetone were carried out in a heated or unheated 250-mL Erlenmeyer flask.

The experiments performed in the autoclave were run at 137^{o}C for a period of 2 hours utilizing an 8% NaOH solution. The analysis of the initial and final material demonstrated that 36.6% of the lignin was removed from the aspen, however no appreciable amount of lignin was removed from the pine. These results confirm those obtained in previous work (1), where it was found that the lignin is more easily removed from the aspen than the pine.

Delignification experiments were performed with aspen utilizing a 4% NaOH solution in both the plug flow reactor and the batch reactor. The results of the experiment, listed in Table no. 6, demonstrated that over 50% of the original cellulose material was lost in the process. In a run performed with pine through the plug flow reactor (162^{o}C, 4.5% NaOH, 11 minute residence time) over 65% of the cellulose material was lost, while 38% of the lignin was removed from the original material.

Experiments were performed with acetone at room temperature and at 55^{o}C. All the experiments were performed for a period of one hour. Up to 19.4% of the lignin was

TABLE NO. 6

DELIGNIFICATION OF EXTRUDED ASPEN
IN 4% NAOH SOLUTION

	BATCH REACTOR	SLR REACTOR
TEMPERATURE (DEG.C.)	165	165
RESIDENCE TIME (MIN)	180	12
%LIGNIN RECOVERED	31.4	53.8
%CELLULOSE LOST	57.1	57.1

found to be removed from the aspen material and up to 8.5% of the lignin was removed from the pine material.

5.5 Hydrolysis of Extruded Material

The results of the hydrolysis runs performed are shown in Table no. 7 (see Appendix IV for detailed mass balances of each run). The feed D.S. ranged from 14.6 to 17.5%. The acid strength of the feed was maintained constant at 0.3% and the pH and % acid/prehydrolyzed wood ranged between 1.5 and 1.6, and 1.5 and 1.8% respectively. The temperature and residence times were varied between 216°C and 226°C, and 1.4 and 2.4 minutes respectively. Yields (% glucose/prehydrolyzed wood) of up to 28.1 were obtained from pine material and yields of up to 32.5 were obtained from aspen. The maximum yield for pine, 28.1%, was obtained at a temperature of 223°C, a residence time of 1.5 minutes and an acid/wood of 1.8%. The maximum yield for aspen, 32.5%, was obtained from runs nos. AH-1, AH-3 and AH-4. Runs nos. AH-1 and AH-4 were run at the same conditions: 220°C, 2.3 minute residence time and 1.5% acid/wood. Run no. AH-3 was run at 226°C, 1.4 minute residence time and 1.6% acid/wood.

The maximum yields obtained based on the %glucose produced as a function of the original wood feed (before the extruder) were 18.7 (pine:PH-3) and 21.9 (aspen:AH-3).

The yields obtained were found to be dependent on the feed material used. The pine feed had a lower cellulose concentration than the aspen feed. As a result the pine runs in general had higher glucose/cellulose yields (up to 65%)

TABLE NO 7A

PINE CELLULOSE HYDROLYSIS

RUN NO	FEED						REACTOR CONDITIONS		PRODUCT				% CONVERSIONS			
	EXTRUDER RUN	D.S. (%)	CELLULOSE /FEED (%)	pH	ACID STRENGTH (%)	ACID /WOOD (%)	RESIDENCE TIME (MIN)	MAXIMUM TEMPERATURE (DEG. C)	D.S. (%)	SOLUBLES (%) (1)	CELLULOSE (%) (1)	LIGNIN (%) (1)	GLUCOSE /FEED (2)	GLUCOSE /CELLULOSE(3)	CELLULOSE (4)	GLUCOSE /WOOD (5)
PH-1	WP-1	14.5	5.6	1.5	0.3	1.6	1.6	220.0	17.2	32.1	27.8	67.1	24.6	57.5	49.6	16.7
PH-2	WP-1	17.2	6.5	1.5	0.3	1.5	1.4	216.0	19.3	32.5	18.0	78.3	24.5	58.1	68.0	16.7
PH-3	WP-1	17.5	6.6	1.5	0.3	1.5	2.4	220.0	19.1	39.9	19.7	76.0	27.5	65.0	68.8	18.7
PH-4	WP-2	14.6	7.4	1.6	0.3	1.8	1.5	223.0	18.1	37.2	17.7	77.6	28.1	49.7	78.2	18.3
PH-5	WP-2	15.9	8.1	1.6	0.3	1.6	2.1	223.0	19.2	33.9	21.0	75.7	25.9	45.8	72.7	16.9
PH-6	WP-3	17.2	8.8	1.6	0.3	1.5	1.7	223.0	19.7	40.3	22.1	72.7	27.2	47.6	73.2	18.2

TABLE NO 7B

ASPEN CELLULOSE HYDROLYSIS

RUN NO	FEED						REACTOR CONDITIONS		PRODUCT				% CONVERSIONS			
	EXTRUDER RUN	D.S. (%)	CELLULOSE /FEED (%)	pH	ACID STRENGTH (%)	ACID /WOOD (%)	RESIDENCE TIME (MIN)	MAXIMUM TEMPERATURE (DEG. C)	D.S. (%)	SOLUBLES (%) (1)	CELLULOSE (%) (1)	LIGNIN (%) (1)	GLUCOSE /FEED (2)	GLUCOSE /CELLULOSE(3)	CELLULOSE (4)	GLUCOSE /WOOD (5)
AH-1	WA-1	15.6	10.2	1.5	0.3	1.5	2.3	220.0	17.0	40.4	38.2	53.6	32.5	44.2	64.1	19.9
AH-2	WA-3	17.4	10.4	1.6	0.3	1.5	1.4	219.0	19.1	30.3	50.6	39.1	22.7	33.8	40.1	15.3
AH-3	WA-3	15.7	9.4	N/A	0.3	1.6	1.4	226.0	18.7	45.7	35.6	54.3	32.5	48.3	66.7	21.9
AH-4	WA-4	16.3	10.0	N/A	0.3	1.5	2.3	220.0	19.1	52.2	43.1	49.5	32.5	47.3	65.3	21.5
AH-5	WA-5	16.8	11.3	1.5	0.3	1.5	1.9	220.0	19.9	39.9	50.8	41.7	26.4	35.1	52.9	17.4

NOTE:
1. Expressed as a percentage of dry product
2. Washed prehydrolyzed material
3. Refers to the amount of glucose produced as a function of the cellulose in the feed material
4. (cellulose in feed - cellulose in product)/cellulose in feed
5. Original wood material

and lower glucose/wood yields (up to 28.1%) than the aspen runs. The glucose/cellulose yields for aspen could have been increased had the cellulose concentration been reduced by utilizing a lower dry solids content in the feed. In the previous work performed with the plug flow reactor (1), a glucose/cellulose yield of 53.9% was obtained utilizing a cellulose feed concentration of 4.2%.

The effect of the cellulose concentration on the glucose/cellulose yield obtained is also demonstrated by the pine runs. The first three pine runs were performed with a cellulose concentration ranging from 5.6 to 6.6%. The yields obtained from those runs ranged between 57.5 and 65.0%. In the next three runs the cellulose concentration was increased to between 7.4 and 8.8%. The yields obtained subsequently dropped to between 45.8 and 49.7%, respectively.

It was noted during experimentation that the lignin contained in the feed caused fouling to occur on the reactor tubes. The fouling was more pronounced during experimentation with aspen than with pine. However, it is believed that in a commercial process fouling would be minimized by providing an adequate velocity to the feed material.

5.6 Washing of Plug Flow Hydrolyzed Material

The material obtained from the plug flow reactor was very viscous, containing small particles of lignin rich wood residue. The washing of this material was performed through both the clarifier/decanter and the de-sludger/clarifier. The material was generally diluted, 1:1 with water, to permit the pumping of the material to either of the solid/liquid separators.

Table no. 8 lists the mass balances that were performed during the solid/liquid separation of both the aspen and the pine. Wash no. WCP was performed with pine hydrolysate utilizing the de-sludger/clarifier. The results obtained demonstrated that 73.3% of the solubles were removed from the original material in a one pass washing process.

A two pass washing system was tested with aspen hydrolysate utilizing the clarifier/decanter (Wash no. WDA). It was determined that in a two pass washing system 93.6% of the soluble material contained in the feed material could be recovered. The washing efficiency was 75.6% and 73.7% respectively for the first and second wash stages.

No particulate material was detected to have been lost during the aspen washing, while approximately 5% was lost during the washing of the pine material.

TABLE NO. 8

WASHING OF HYDROLYZED MATERIAL: CLARIFIER/DECANTER; DE-SLUDGER/CLARIFIER

WASH NO	MATERIAL	PASS NO	%D.S. FEED	%SOLUBLES FEED	%D.S. UNDERS	%INSOLUBLES LOST IN OVERS	%SOLUBLES REMOVED	%SOLUBLES REMOVED OVERALL
WCP (1)	PINE	1	11.4	46.2	20.1	4.0	73.3	73.3
WDA (2)	ASPEN	1	8.3	33.2	23.0	-	75.7	75.7
	ASPEN	2	5.2	11.8	21.6	-	73.7	93.6

NOTE: (1). WCP PERFORMED WITH DE-SLUDGER/CLARIFIER

(2). WDA PERFORMED WITH CLARIFIER/DECANTER

5.7 Fermentation

Pure sugar streams (xylose and glucose), and prehydrolysate and hydrolysate streams obtained from both aspen and pine were fermented at the University of Toronto and Bio-Hol. This work was performed under subcontract by Professor Morris Wayman. A detailed description of the work performed and the results obtained from this work are contained in the report submitted by Professor Wayman which is enclosed in Appendix V.

Table no. 9 lists the results of the fermentations performed. All the fermentations used *Pichia stipitis R* yeast except Run no. 5 which used *Saccharomyces cerevisiae*. The fermentations were performed in either a 4-L or 28-L fermentor. Yeast extract, malt extract and peptone were used as the nutrients for all fermentations. The fermentation times varied between 12 and 92 hours. The acetic acid concentration of all the prehydrolysate streams used was decreased to below 0.5-g/L through steam stripping before being fermented.

Fermentations were performed with pure xylose solutions in both the 4-L and 28-L fermentors, in Runs nos. 1 and 2 respectively. The yields (Yp/s: g-ethanol/g-sugar produced) of the two runs were 0.485 (Run no. 1) and 0.44 (Run no. 2). The sugar consumed ranged between 94.6% (Run no.1) and 94.3% (Run no. 2).

TABLE NO 9

FERMENTATION RESULTS

RUN NO	FERMENTER SIZE (L)	WORKING VOLUME (L)	SUBSTRATE	NUTRIENT (Y;M;P) CONCENTRATION (g/L) (1)	YEAST	INITAL SUGAR (g/L)	INITIAL BIOMASS (g/L)	FERMENTATION TIME (HRS)	FINAL ETHANOL (g/L)	FINAL SUGAR (g/L)	SUGAR CONSUMED (%)	Yx/s (2)	Yp/s (3)	Vp (4)	Qp (5)
1	4	2.7	XYLOSE	2;2;3	Pichia stipitis R	28.0	1.4	32	13.0	1.5	94.6	0.19	0.485	0.40	0.076
2	28	17.0	XYLOSE	2;2;3	Pichia stipitis R	56.6	2.8	64	23.4	3.2	94.3	0.24	0.44	0.37	0.035
3	28	20.0	GLUCOSE/ XYLOSE	2;2;3	Pichia stipitis R	130.0	0.4	92	55.0	7.0	94.6	0.10	0.445	0.59	0.050
4	28	15.0	ASPEN PREHYD (5)	3;3;5	Pichia stipitis R	60.0	9.0	68	24.0	6.0	90.0	0.06	0.44	0.35	0.10
5	4	2.7	ASPEN HYD (6)	1;1;1	Saccharomyces cerevisiae	103.0	14.0	68	43.0	12.0	88.3	0.05	0.477	0.63	0.15
6	28	15.0	ASPEN PRE/ HYD	1;1;2	Pichia stipitis R	116.0	10.0	66	46.5	4.0	96.6	0.13	0.43	0.70	0.14
7	4	2.7	PINE PRE	1;1;2	Pichia stipitis R	53.0	16.7	41	23.5	5.0	90.6	0.11	0.49	0.56	0.10
8	4	2.7	PINE HYD	1;1;1	Pichia stipitis R	78.5	14.0	12	35.0	1.5	98.0	0.04	0.45	2.90	0.85
9	4	2.7	PINE PRE/ HYD	1;1;2	Pichia stipitis R	102.0	8.0	36	45.3	5.0	95.1	0.06	0.45	1.29	0.21

NOTE: (1). Y;M;P (g/L): YEAST EXTRACT (g/L); MALT EXTRACT (g/L); PEPTONE (g/L)

(2). Yx/s: g yeast / g sugar consumed

(3). Yp/s: g ethanol/ g sugar consumed

(4). Vp : g ethanol/ L-hr

(5). Qp : g ethanol/ g biomass(final)-hr

(6). PRE: PREHYDROLYSATE

(7). HYD: HYDROLYSATE

Run no. 3 was performed with a sugar mixture (30% xylose, 70% glucose), simulating the sugar mixture to be expected if the prehydrolysate and hydrolysate were mixed together. The fermentation time for this run was 92 hours and a Yp/s of 0.445 was obtained.

The next series of runs were performed with aspen prehydrolysate (Run no. 4), aspen hydrolysate (Run no. 5) and a mixture of aspen prehydrolysate and hydrolysate (Run no 6). The fermentation of the prehydrolysate was performed with an initial sugar concentration of 60-g/L, while both the hydrolysate and mixture fermentations were performed with an initial sugar concentration of greater than 100-g/L. The highest Yp/s (0.477) was obtained from the fermentation of the hydrolysate with Saccharomyces cerevisiae. The final ethanol concentration obtained ranged between 24.0-g/L (Run no. 4) and 46.5-g/L (Run no.6).

The final series of runs were performed with streams obtained through pine hydrolysis and prehydrolysis. The initial sugar concentration of these runs ranged between 53.0-g/L (Run no. 7) and 102-g/L (Run no. 9). The yield obtained ranged between 0.49 (Run no. 7) and 0.45 (Runs nos. 8 and 9).

Comparing the fermentations of pine and aspen streams it can be concluded that the pine streams fermented much more readily than the aspen streams. The maximum Vp (g-ethanol/L-hr) obtained from the pine fermentations was over four times the maximum obtained with aspen. The Yp/s values obtained from the pine streams were also higher than those obtained from the aspen streams in two (Run no. 9 versus 6 and Run no. 7 versus 4) out of the three runs.

6.0 OVERALL PROCESS FLOWSHEET AND ECONOMIC EVALUATION

The economic evaluations presented in this section are based on a wood to ethanol plant integrated with a pulp and paper mill based in Northern Ontario. The figures used for this evaluation are based on data obtained from the E.B. Eddy pulp and paper mill located in Espanola, Ontario. All costs are expressed in Canadian dollars.

6.1 Quantity and Value of Feedstock

The waste feedstock available at the plant is approximately 210 000 MT/yr of primarily pine wood at 52% D.S.. Presently, the plant is using the waste wood to generate steam and electricity. The present value of the waste wood is $46.20/ODMT based on its fuel value equivalent.

6.2 Overall Mass Balance and Unit Processing Cost

The overall mass balance of the plant is shown in Table no. 10. Based on the amount of waste wood available a 20,000,000 L/yr ethanol plant was designed. The flowsheet of the proposed plant, which corresponds to the streams presented in Table no. 10, is shown in Figure no. 3.

TABLE NO. 10

MASS BALANCE : PINE TO ETHANOL PLANT - 20 000 000 L/yr

HIGHLIGHTS	STREAM	1	2	3	4	5	6	7	8
	NAME	WOOD FEED	H2SO3 FEED	EXTRUDER PRODUCT (B)	FLASHED PRODUCT @ 20 PSIG	STEAM FLASH @ 20 PSIG	FLASHED PRODUCT @ 0 PSIG	STEAM FLASH @ 0 PSIG	PROCESS WATER TO SCREENS
ANNUAL PRODUCTION	CELLULOSE	86.91	0.00	67.18	67.18	0.00	67.18	0.00	0.00
20000000	HEMICELLULOSE	36.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	LIGNIN	61.30	0.00	59.46	59.46	0.00	59.46	0.00	0.00
PROCESS YIELD L/MT	GLUCOSE	0.00	0.00	11.15	11.15	0.00	11.15	0.00	0.00
216.96	XYLOSE	0.00	0.00	33.58	33.58	0.00	33.58	0.00	0.00
	ARABINOSE	0.00	0.00	3.08	3.08	0.00	3.08	0.00	0.00
% RECYCLE TO SLR	ACETIC ACID	0.00	0.00	2.39	2.39	0.00	2.39	0.00	0.00
0.00	FURFURAL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	ETHANOL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	OTHER (A)	8.92	2.13	27.86	27.86	0.00	27.86	0.00	0.00
	WATER	179.07	40.54	211.03	181.69	29.34	167.04	14.65	668.18
	TOTAL WEIGHT	373.06	42.68	415.74	386.39	29.34	371.75	14.65	668.18
	D.S. (%)	52.00	5.00	49.24	52.98	0.00	55.06	0.00	0.00
	TOTAL D.S.	193.99	2.13	204.70	204.70	0.00	204.70	0.00	0.00
	SOLUBLES(%)	0.00	100.00	35.00	35.00	0.00	35.00	0.00	0.00
	INSOLUBLES(%)	100.00	0.00	65.00	65.00	0.00	65.00	0.00	0.00
	USGPM								
	TEMP(DEG.F.)			347.00	259.00	259.00	212.00	212.00	
	ENTHALPY(BTU/KG)			522.39	367.12	2567.10	281.86	2530.90	
	C.P.			1.66	1.62		1.59		

NOTE:
Where not specified units are in kg/min
A. To reduce the number of elements listed,
the less important elements are grouped as others.
B. Based on the results obtained from run WP-2.

TABLE NO. 10 (CONTINUED)

STREAM	9	10	11	12	13	14	15	16	17	18
NAME	LIQUID FROM SCREENS (C)	SOLIDS FROM SCREENS (C)	SOLIDS TO SLR	HCl FEED REACTOR	WATER FOR FEED	FEED SLR REACTOR	PRODUCT SLR REACTOR (D)	STEAM SLR @ 100 PSIG	STEAM SLR @ 345 PSIG	FLASH SLR @ 20 PSIG
CELLULOSE	6.72	60.46	60.46	0.00	0.00	60.46	13.18	0.00	0.00	0.00
HEMICELLULOSE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LIGNIN	5.95	53.52	53.52	0.00	0.00	53.52	53.52	0.00	0.00	0.00
GLUCOSE	10.49	0.67	0.67	0.00	0.00	0.67	34.86	0.00	0.00	0.00
XYLOSE	31.56	2.01	2.01	0.00	0.00	2.01	0.00	0.00	0.00	0.00
ARABINOSE	2.90	0.19	0.19	0.00	0.00	0.19	0.00	0.00	0.00	0.00
ACETIC ACID	2.24	0.14	0.14	0.00	0.00	0.14	0.00	0.00	0.00	0.00
FURFURAL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ETHANOL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OTHER	20.80	7.06	7.06	2.23	0.00	9.29	26.41	0.00	0.00	0.00
WATER	545.78	289.45	289.45	4.74	322.36	616.55	614.87	206.58	81.15	125.81
TOTAL WEIGHT	626.43	413.50	413.50	6.98	322.36	742.83	742.83	206.58	81.15	125.81
D.S. (%)	12.87	30.00	30.00	32.00	0.00	17.00	17.23	0.00	0.00	0.00
TOTAL D.S.	80.65	124.05	124.05	2.23	0.00	126.28	127.96	0.00	0.00	0.00
SOLUBLES(%)	83.50	3.47	-	100.00	-	5.17	37.20	0.00	0.00	0.00
INSOLUBLES(%)	16.50	96.53	-	0.00	-	94.83	62.80	0.00	0.00	0.00
USGPM						192.44				
TEMP(DEG.F.)							433.00	338.00	433.00	259.00
ENTHALPY(BTU/KG)							806.22	1937.50	1748.34	2567.10
C.P.							2.01			

NOTE:
Where not specified units are in kg/min
C. Based on the mass balance obtained from run WSPP-1
and assuming 5% of the insolubles pass though the screens in each pass.
D. Based on the results from run no. PH-4

TABLE NO. 10 (CONTINUED)

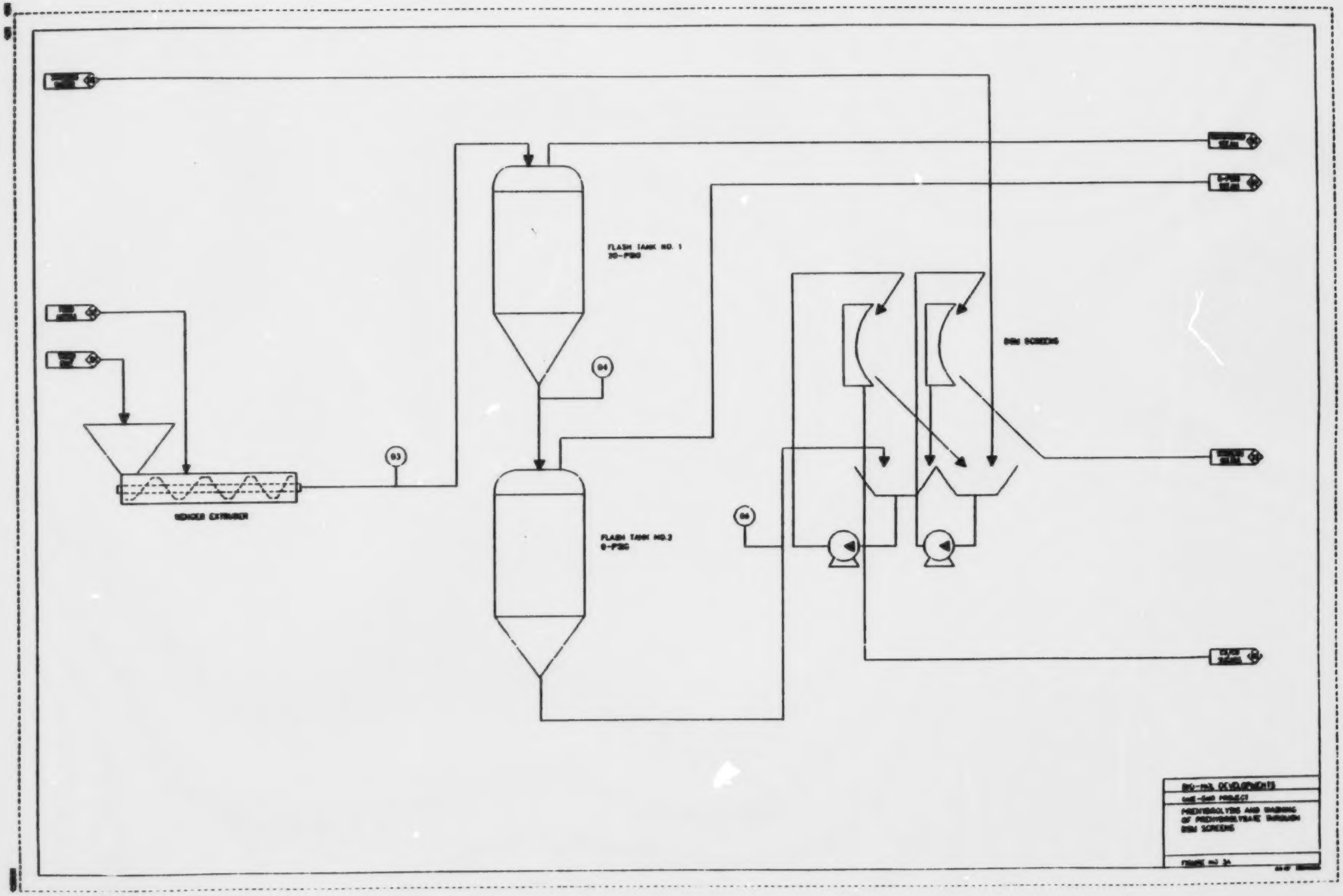
STREAM	19	20	21	22	23	24	25	26	27	28
NAME	PRODUCT SLR @ 20 PSIG	PRODUCT SLR @ 0 PSIG	FLASH SLR @ 0 PSIG	PROCESS WATER CENTRIFUGE	SOLIDS CENTRIFUGE (E)	SOLIDS RECYCLED	LIQUID CENTRIFUGE (E)	STEAM STRIPPED	STRIPPING STEAM	SUGAR STREAM
CELLULOSE	13.18	13.18	0.00	0.00	12.13	0.00	1.05	6.72	0.00	7.77
HEMICELLULOSE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LIGNIN	53.52	53.52	0.00	0.00	49.23	0.00	4.28	5.95	0.00	10.23
GLUCOSE	34.86	34.86	0.00	0.00	2.19	0.00	32.67	10.49	0.00	43.16
XYLOSE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.56	0.00	31.56
ARABINOSE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.90	0.00	2.90
ACETIC ACID	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.00	0.39
FURFURAL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ETHANOL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
OTHER	26.41	26.41	0.00	0.00	13.71	0.00	12.69	20.80	0.00	33.49
WATER	489.07	462.33	26.74	462.33	307.13	0.00	617.53	545.78	95.06	1163.31
TOTAL WEIGHT	617.03	590.29	26.74	462.33	384.39	0.00	668.23	624.57	95.06	1292.80
D.S. (%)	20.74	21.68	0.00	0.00	20.10	20.10	7.59	12.62	0.00	10.02
TOTAL D.S.	127.96	127.96	0.00	0.00	77.26	0.00	50.70	78.80	0.00	129.50
SOLUBLES(%)	37.20	37.20	0.00	0.00	4.31	4.31	87.32	83.50	-	86.19
INSOLUBLES(%)	62.80	62.80	0.00	0.00	95.69	95.69	12.68	16.50	-	13.81
USGPM										
TEMP(DEG.F.)	259.00	212.00	212.00						212.00	
ENTHALPY(BTU/KG	447.19	352.80	2530.90						1937.50	
C.P.	1.97	1.96								

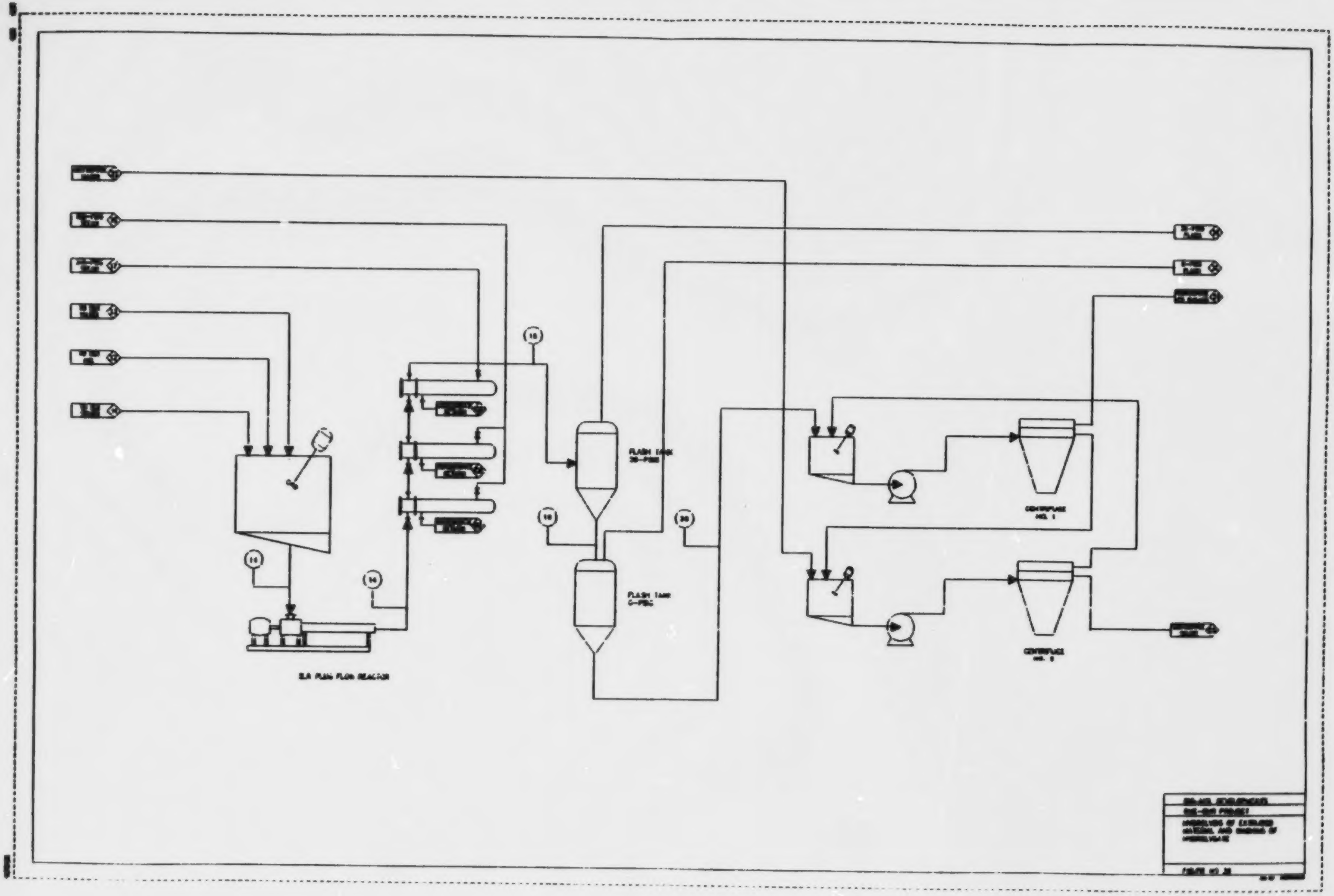
NOTE:
Where not specified units are in kg/min
E. Based on the results obtained from wash no. WCP

TABLE NO. 10 (CONTINUED)

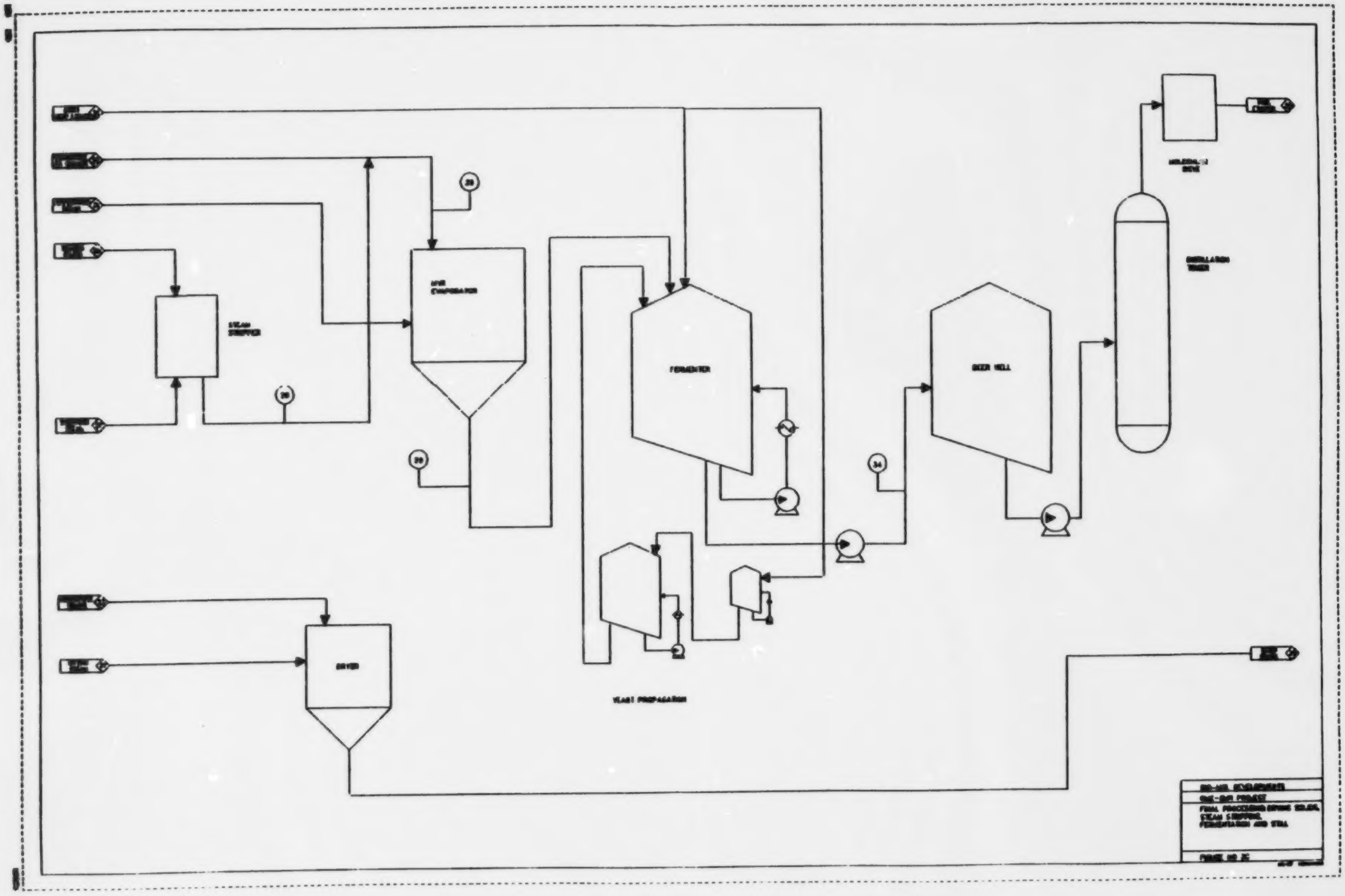
STREAM	29	30	31	32	33	34	35	36	37
NAME	CONCENTRATED SUGAR	EVAPORATION STREAM	STEAM EVAPORATION	CORN STEEP LIQUOR	FEED TO FERMENTORS	FERMENTOR PRODUCT (F)	DISTILLATION PRODUCT	DRIED SOLIDS CENTRIFUGED	STEAM FOR DRYING
CELLULOSE	7.77	0.00	0.00	0.00	7.77	7.77	0.00	12.13	0.00
HEMICELLULOSE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LIGNIN	10.23	0.00	0.00	0.00	10.23	10.23	0.00	49.23	0.00
GLUCOSE	43.16	0.00	0.00	0.00	43.16	0.00	0.00	2.19	0.00
XYLOSE	31.56	0.00	0.00	0.00	31.56	3.66	0.00	0.00	0.00
ARABINOSE	2.90	0.00	0.00	0.00	2.90	1.65	0.00	0.00	0.00
ACETIC ACID	0.39	0.00	0.00	0.00	0.39	0.39	0.00	0.00	0.00
FURFURAL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ETHANOL	0.00	0.00	0.00	0.00	0.00	33.38	32.71	0.00	0.00
OTHER	33.49	0.00	0.00	3.88	43.58	82.51	0.00	13.71	0.00
WATER	611.34	551.97	16.67	0.43	636.61	636.61	0.33	71.32	336.87
TOTAL WEIGHT	740.84	551.97	16.67	4.31	776.20	776.20	33.04	148.58	336.87
D.S. (%)	17.48	0.00	0.00	90.00	17.98	17.98	99.00	52.00	0.00
TOTAL D.S.	129.50	0.00	0.00	3.88	139.59	139.59	32.71	77.26	0.00
SOLUBLES(%)	86.19	-	-	100.00	-	-	100.00	4.31	-
INSOLUBLES(%)	13.81	-	-	0.00	-	-	0.00	95.69	-
USGPM									
TEMP(DEG.F.)									
ENTHALPY(BTU/KG)									
C.P.									

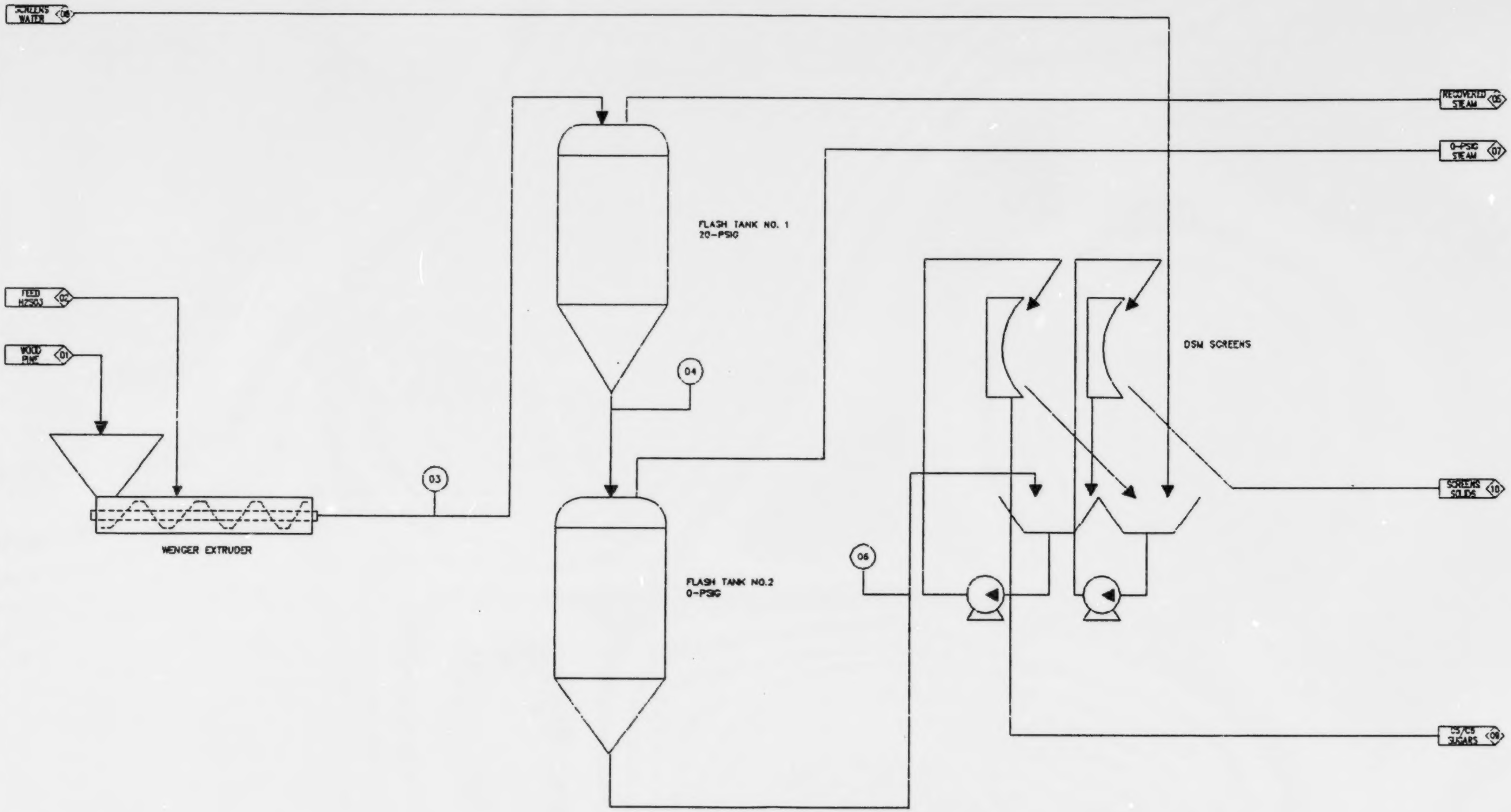
NOTE:
Where not specified units are in kg/min
F. Based on fermentor run no. 9

FLASH TANK NO. 1
30-PSIG
FLASH TANK NO.2
0-PSIG
DSM SCREENS
WENGER EXTRUDER
03
04
06
BIO-HOL DEVELOPMENTS
PREHYDROLYSIS AND WASHING
OF PREHYDROLYSATE THROUGH
DSM SCREENS
FIGURE NO 3c



FLASH TANK
30-PSIG
FLASH TANK
0-PSIG
CENTRIFUGE
NO. 1
CENTRIFUGE
NO. 2
3.5 PLUG FLOW REACTOR
15
16
30
HYDROLYSIS OF EXTRUDED MATERIAL AND WASHING OF HYDROLYSATE


STEAM STRIPPER
26
MVR EVAPORATOR
28
30
FERMENTER
YEAST PROPAGATION
34
BEER WELL
DISTILLATION TOWER
MOLECULAR SIEVE
DRYER

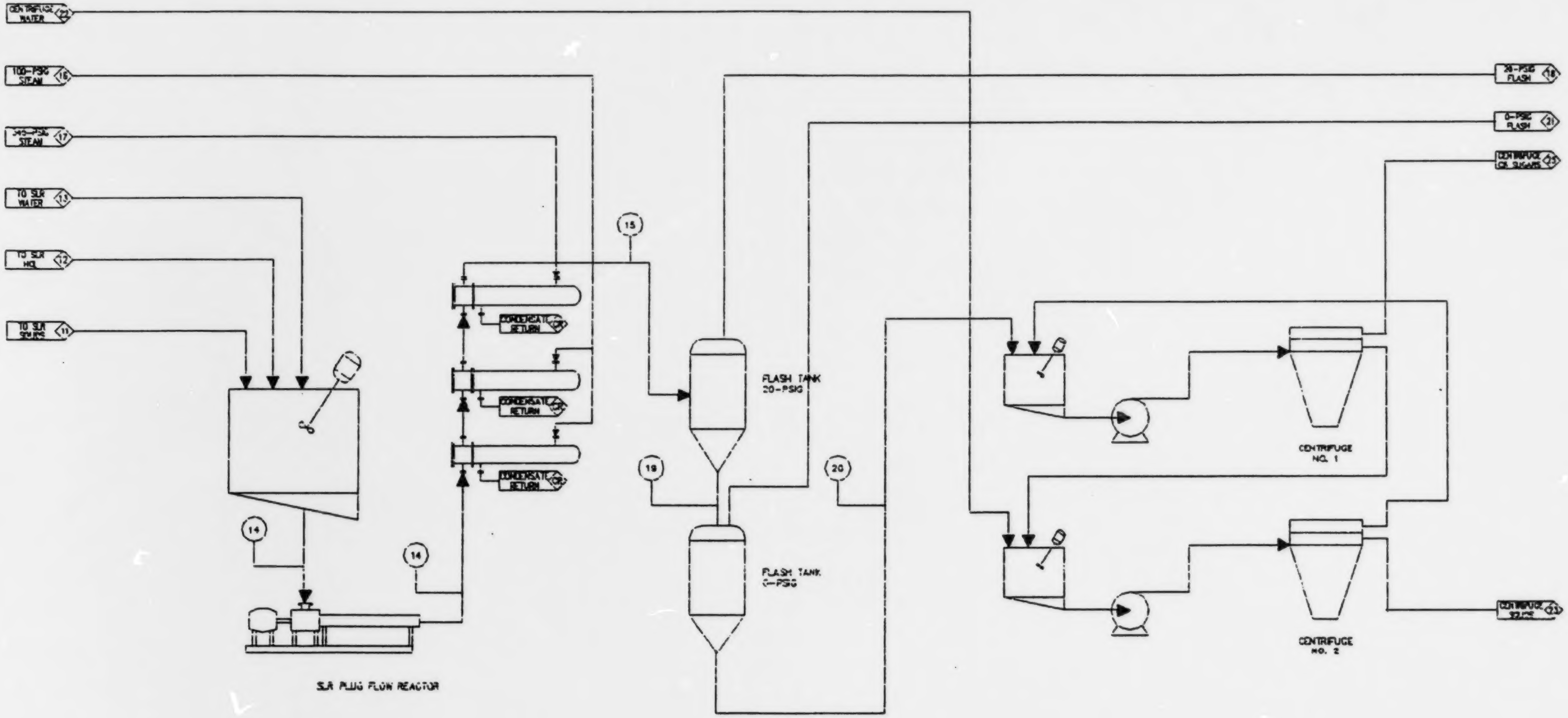


BIO-HOL DEVELOPMENTS

OME-EMR PROJECT

PREHYDROLYSIS AND WASHING
OF PREHYDROLYSATE THROUGH
DSM SCREENS

FIGURE NO 3A

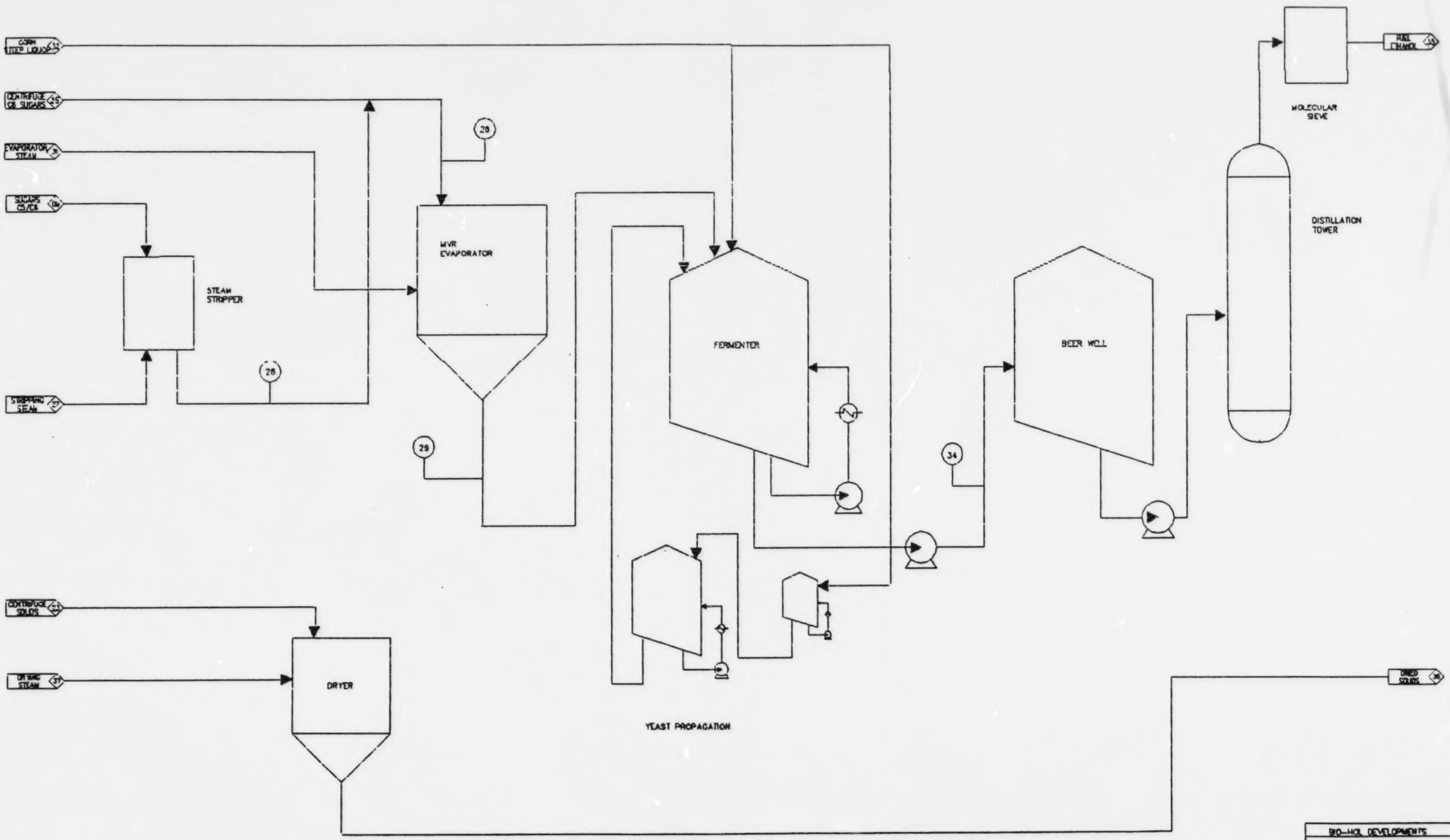


BIO-HOL DEVELOPMENTS

OME-EMR PROJECT

HYDROLYSIS OF EXTRUDED
MATERIAL AND WASHING OF
HYDROLYSATE

FIGURE NO 38



BIO-HOL DEVELOPMENTS

OME-DIR PROJECT

FINAL PROCESSING:DRYING SOLIDS, STEAM STRIPPING, FERMENTATION AND STILL

FIGURE NO 3C

6.2.1 Prehydrolysis

The yields used for prehydrolysis are based on run no. WP-2. The installed capital cost of each Wenger extruder unit is estimated at $ 786,000 (2) (including controls). Each unit is capable of processing 10,000-lbs/hr of wet wood. Based on the mass balance, six Wenger extruders would be required, resulting in a capital cost of $4,716,000, including instrumentation and controls. The system would also require two flash tanks (20-psig,0-psig) with an estimated cost of $100,000. The overall cost of the system would therefore be $4,816,000.

The electrical consumption of the extruder is estimated at 80 kWh/ODMT.

6.2.2 Washing of prehydrolysate material

The washing of the prehydrolysate material will be accomplished on 120° DSM screens produced by Dorr-Oliver (see Appendix VI for description of screens). This system was chosen over filter presses and belt presses due to the lower capital cost involved.

The washing system is a two stage countercurrent system. The washing efficiency is estimated to be equivalent to that achieved with a two stage filter press system, 94.0% (solubles removed). The final dry solids content is estimated at 30% and it is estimated that 5% of the insolubles would be lost in each overs stream. The cost of the screens alone is $83,000 (3). The installed cost of the system is estimated at $205,500. Instrumentation and controls for the system are estimated at 3% of the installed capital cost resulting in a total cost of $211,665.

6.2.3 Steam stripping

The installed cost including controls of the steam stripper is estimated at $25,000. The more significant cost involved in this processing unit is the steam cost. The steam cost is estimated as the energy needed to heat the incoming stream to 100°C.

6.2.4 SLR plug flow reactor

Based on the mass balance one 200-USGPM SLR plug flow reactor unit will be required. The estimated installed cost of the unit including instrumentation and controls is $1,500,000 . Based on the experiments performed the reactor will process feeds of 17% D.S. The yields used are based on run PH-4.

The product from the plug flow reactor will pass through a 20-psig flash tank in order to recover some steam. The estimated cost of the flash tanks unit is $200,000. The overall cost of the plug flow system is estimated at $1,700,000.

6.2.5 Washing and solids/liquid separation of hydrolyzed material

The hydrolyzed material will be washed in a countercurrent washing system utilizing two Westfalia Desludger/Clarifiers (see Appendix VII for equipment specifications). The cost per unit (SA 160-06-117) is $800,000 (4). The installation, instrumentation and controls cost is estimated at 20% of the capital cost, resulting in an overall cost for the system of $1,920,000.

6.2.6 Concentration of the sugars streams

The sugars stream will be mixed together and concentrated to 105-g/L. The evaporator to be used is an MVR (mechanical vapour recompression) unit. It is estimated that the installed cost of the unit will be $1,063,000 (5) (including instrumentation and controls). The utility costs of the system are estimated at 200lbs water evaporated/hp-hr for the electrical and 1000 kg/hr of steam.

6.2.7 Fermentation system

The conditions of the fermentation stream are based on the results obtained from fermentation run no. 9. It has been assumed that corn steep liquor can be successfully substituted for YMP as the nutrient for the fermentation.

It is estimated that the fermentation cycle will be 48 hours and that 4 - 175,000 US gallon tanks plus one additional tank (beer well) of the same capacity will be required. The installed cost of the fermentation tanks plus the beer well is estimated at $925,000 (6). Each fermentor will be equipped with a heat exchanger and a recirrculation pump. In addition, a drain pump interconnected to all the fermentors will be provided. The estimated cost of the additional equipment is $102,000.

The cost of yeast propagation is estimated at $120,000. An additional cost of $20,000 is estimated for the CIP (clean in place) system. The total installed cost of the fermentation system including instrumentation and controls (4% of the installed equipment cost) is estimated at $1,213,680.

6.2.8 Distillation column and molecular sieve

The cost of the distillation column and the molecular sieve based on the production of 20,000,000 L/yr of 99.9% ethanol is $1,600,000 (7) installed including instrumentation and controls. The utility cost associated with the unit is estimated at 6.7-lbs steam per liter of ethanol produced.

6.2.9 Dryer for lignin rich residue

The estimated installed capital cost of the drying unit is $305,800 (8) (including instrumentation and controls). The efficiency of the dryer is estimated at 70%.

6.2.10 Overall plant yield

The overall ethanol yield for the plant obtained from Table no. 10 was 217-L/ODMT. To improve the yield the effect of a recycle into the plug flow reactor was investigated. The results obtained from the model demonstrated that a 50% recycle into the plug flow reactor would increase the ethanol yield to 229.2-L/ODMT. It was noted however that the operating cost rose from $0.4468/L (no recycle) to

$0.4485/L, when a 50% recycle was employed. This increase in operating cost was due primarily to the steam cost associated with the plug flow reactor, which rose approximately $0.01/L in the recycle process. It is believed that the recycling of the processed material is not economical due its low cellulose content (<20%). This demonstrates the need to successfully isolate the cellulose contained in the product from the plug flow reactor before recycling, in order to render recycling economical. It is important to note that the research to successfully isolate the cellulose fraction is now presently being pursued at Bio-Hol.

In the previous work performed (1) on a wood to ethanol process incorporating the plug flow reactor for both prehydrolysis and hydrolysis, it was estimated that the maximum yield obtainable was 314-L/ODMT. The difference between the yield obtained in this study and that obtained previously was due primarily to: the consideration of washing efficiencies; lower yield obtained through the extruder than the plug flow reactor for prehydrolysis; the elimination of a recycle stream. In the previous work no losses were taken into account for the two washing stages. It was determined in this study that approximately 20-L/ODMT would not be recovered in the washing. The lower yield obtained through the Wenger extruder resulted in a difference of 40-L/ODMT between the yields obtained from the two processes. Finally, the fact that no recycle was employed in this study resulted in a difference of approximately 40-L/ODMT with the projections made in the previous study.

6.3 Plant Cost

6.3.1 Fixed capital investment

Table no. 11 lists the capital equipment costs outlined in section 6.2. The total fixed capital investment for the plant was estimated at $18,511,409, based on a total equipment cost estimate of $12,855,145.

6.3.2 Operating costs

The projected utility and chemical costs associated with each processing unit are outlined in Table no 12. Table no 13 outlines the total operating cost projected for the plant. The overall cost was estimated at $0.4468/L or $8,936,356/yr based on a 20,000,000-L/yr plant. The major operating costs for the plant are for steam and for raw materials.

6.3.3 IRR sensitivity analysis

Based on the costs and capital investment presented, an IRR of -27.22% was calculated assuming a selling price for ethanol of $0.40/L.

TABLE NO. 11

TOTAL FIXED CAPITAL INVESTMENT

PROCESS	EQUIPMENT	COST
PREHYDROLYSIS	WENGER EXTRUDER	$4,816,000
WASHING PREHYDROLYSATE	DSM SCREENS	$211,665
REMOVAL OF ACETIC ACID	STEAM STRIPPER	$25,000
HYDROLYSIS	SLR REACTOR	$1,700,000
WASHING HYDROLYSATE	CENTRIFUGES	$1,920,000
CONCENTRATION OF SUGARS	EVAPORATER	$1,063,000
FERMENTATION	FERMENTERS	$1,213,680
DISTILLATION	STILL/MOLECULAR SIEVE	$1,600,000
DRYING WOOD RESIDUE	DRYER	$305,800
TOTAL EQUIPMENT COST		$12,855,145
TOTAL INDIRECT COST (15% OF EQUIPMENT)		$1,928,272
PIPING AND ELECTRICAL (10%)		$1,285,515
LAND (1%)		$128,551
BUILDING (13%)		$1,671,169
WORKING CAPITAL (5%)		$642,757
TOTAL FIXED CAPITAL COST		$18,511,409

TABLE NO 12

UTILITY AND CHEMICAL COSTS OF THE UNIT PROCESSES

UTILITIES COST	PROCESSING UNIT									
	WENGER EXTRUDER		STEAM STRIPPING		PLUG FLOW		SEPARATION		EVAPORATION	
	CENTS PER LITER	ANNUAL COST ($)	CENTS PER LITER	ANNUAL COST ($)	CENTS PER LITER	ANNUAL COST ($)	CENTS PER LITER	ANNUAL COST ($)	CENTS PER LITER	ANNUAL COST ($)
ELECTRICAL @ $0.0365/kWH	1.78	355050.06	-	-	-	-	0.50	100000.00	0.39	79480.21
STEAM @ $4.46/1000LBS-100PSIG	-	-	2.22	443211.62	4.82	963221.69	-	-	0.39	77711.04
STEAM @ $6.54/1000LBS-345PSIG	-	-	-	-	2.77	554847.68	-	-	-	-
CREDIT STEAM FLASH OR WOOD FUEL	0.91	181272.10	-	-	3.89	777206.02	-	-	-	-
PROCESS WATER @ $0.60/1000USG	-	-	-	-	0.12	23788.80	0.42	83426.70	-	-
CHEMICAL COST										
SO2 @ $0.124/lbs	1.38	276625.89	-	-	-	-	-	-	-	-
HCl @ $0.034/lbs-32.0%HCl	-	-	-	-	1.24	248023.87	-	-	-	-
CORN STEEP LIQUOR @ $155.00/MT	-	-	-	-	-	-	-	-	-	-
TOTAL COST	2.25	812948.05	2.22	443211.62	5.06	1012676.01	0.42	83426.70	0.78	157191.25

NOTE:
Utility cost obtained from E.B.Eddy and chemical cost obtained from Chemical Marketing Reporter, January 1989

TABLE NO. 12 (CONTINUED)

UTILITIES COST	PROCESSING UNIT					
	FERMENTATION		DISTILLATION		DRYING LIGNIN RESIDUE	
	CENTS PER LITER	ANNUAL COST ($)	CENTS PER LITER	ANNUAL COST ($)	CENTS PER LITER	ANNUAL COST ($)
ELECTRICAL @ $0.0365/kWH	0.50	100000.00	-	-	-	-
STEAM @ $4.46/1000LBS-100PSIG	-	-	2.99	597640.00	7.85	1570699.70
STEAM @ $6.54/1000LBS-345PSIG	-	-	-	-	-	-
CREDIT STEAM FLASH OR WOOD FUEL	-	-	-	-	8.48	1696228.49
PROCESS WATER @ $0.60/1000USG	0.23	46122.35	-	-	-	-
CHEMICAL COST						
SO2 @ $0.124/lbs	-	-	-	-	-	-
HCl @ $0.034/lbs-32.0%HCl	-	-	-	-	-	-
CORN STEEP LIQUOR @ $155.00/MT	1.43	285857.70	-	-	-	-
TOTAL COST	2.16	431980.05	2.99	597640.00	-0.63	-125528.80

NOTE:
Utility cost obtained from E.B.Eddy and chemical cost obtained from
Chemical Marketing Reporter, January 1989

TABLE NO 13

TOTAL OPERATING COSTS

	CENTS PER LITER	DOLLARS ANNUALLY
ELECTRICAL	3.17	$633,641
STEAM COST	8.39	$1,678,154
PROCESS WATER	0.77	$153,338
CHEMICALS	4.05	$810,507
LABOUR	3.60	$720,000
MAINTENANCE (1)	2.78	$555,342
SUPERVISORY	0.54	$108,000
ADNIMISTRATION	0.72	$144,000
RAW MATERIAL COST	21.29	$4,258,903
LIGNIN RESIDUE CREDIT (2)	-0.63	($125,529)
TOTAL OPERATING COST	44.68	$8,936,356

(1) MAINTANENCE IS ESTIMATED AS 3% OF THE FIXED CAPITAL COST
(2) LIGNIN RESIDUE CREDIT INCORPORATES BOTH THE REVENUE FROM THE RESIDUE AND THE COST FOR DRYING THE RESIDUE.

The effect on the IRR of the price of ethanol, the feedstock cost, the value of the lignin rich residue and the yield respectively, are analyzed in Figures nos. 4-7. Figure no 4 demonstrates that an IRR of 20% could only be accomplished with a selling price for ethanol of approximately $0.70/L. The most encouraging of the sensitivity analysis was presented in Figure no 6, where the effect of the value of residue on the IRR was investigated. This analysis demonstrated that an IRR of 22% could be accomplished if the lignin rich residue had a net value of approximately $0.10/lbs. A cash flow analysis is presented in Table no. 14 assuming a $0.10/lbs value for the lignin rich stream. It is uncertain if the residue stream, which contains approximately 64% lignin, would be able to be sold for $0.10/lbs as is, or if the lignin would have to be purified. Research is now being pursued at Bio-Hol to identify what are the best options available for the lignin rich stream.

FIGURE NO. 4

EFFECT OF ETHANOL PRICE ON IRR

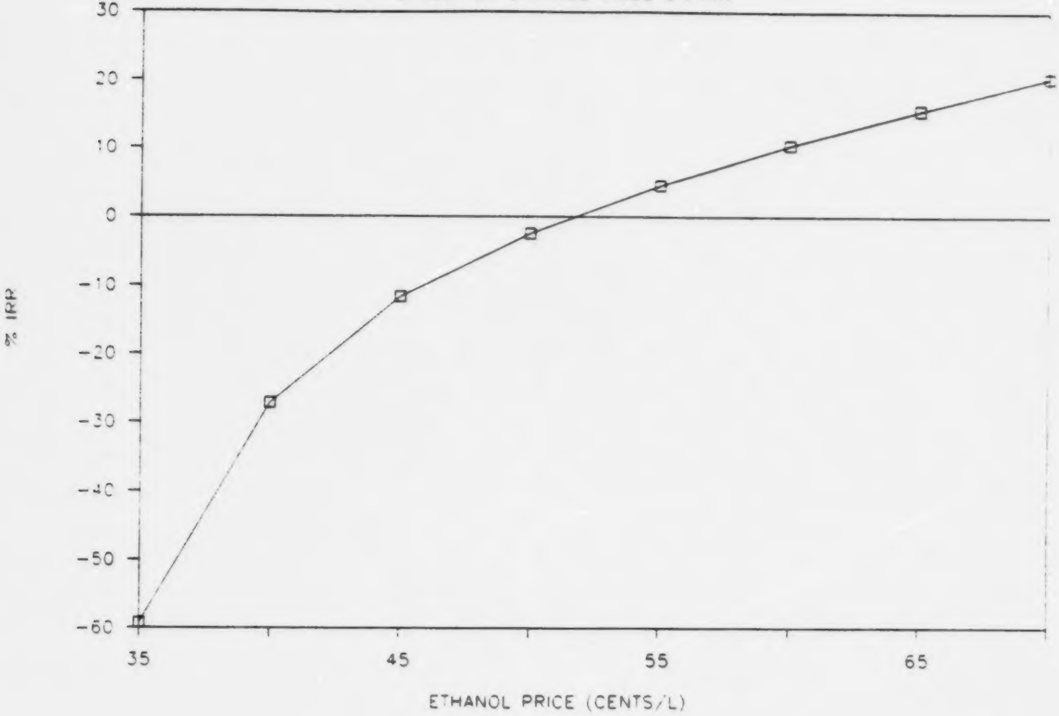


FIGURE NO. 5

EFFECT OF FEEDSTOCK COST ON IRR

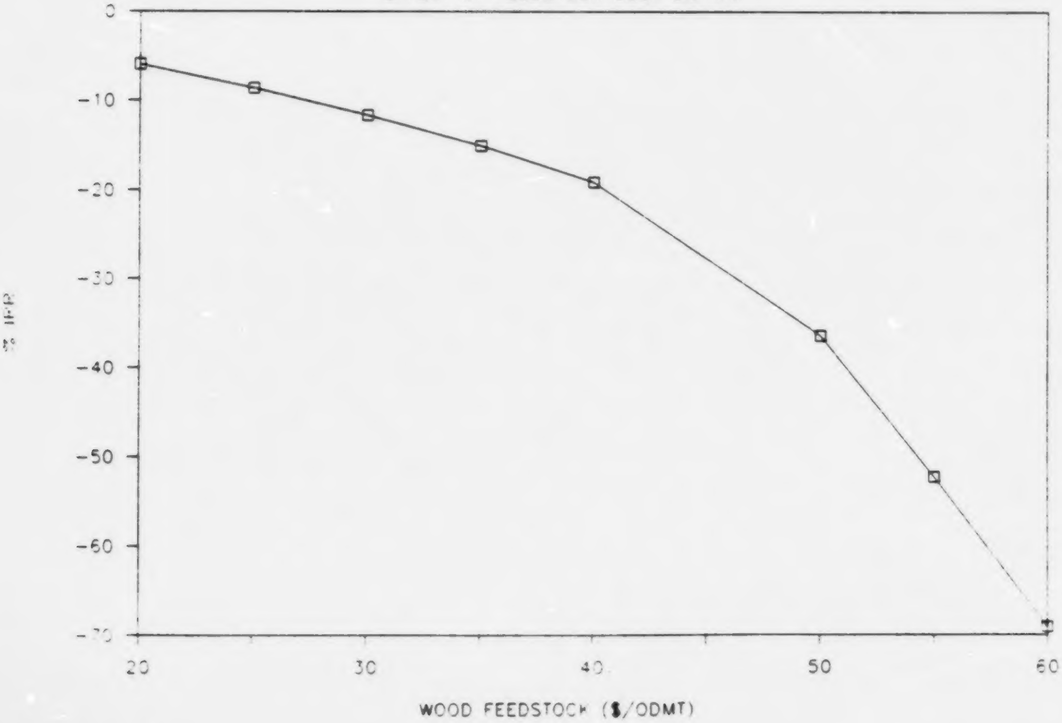


FIGURE NO. 6

EFFECT OF LIGNIN VALUE ON IRR

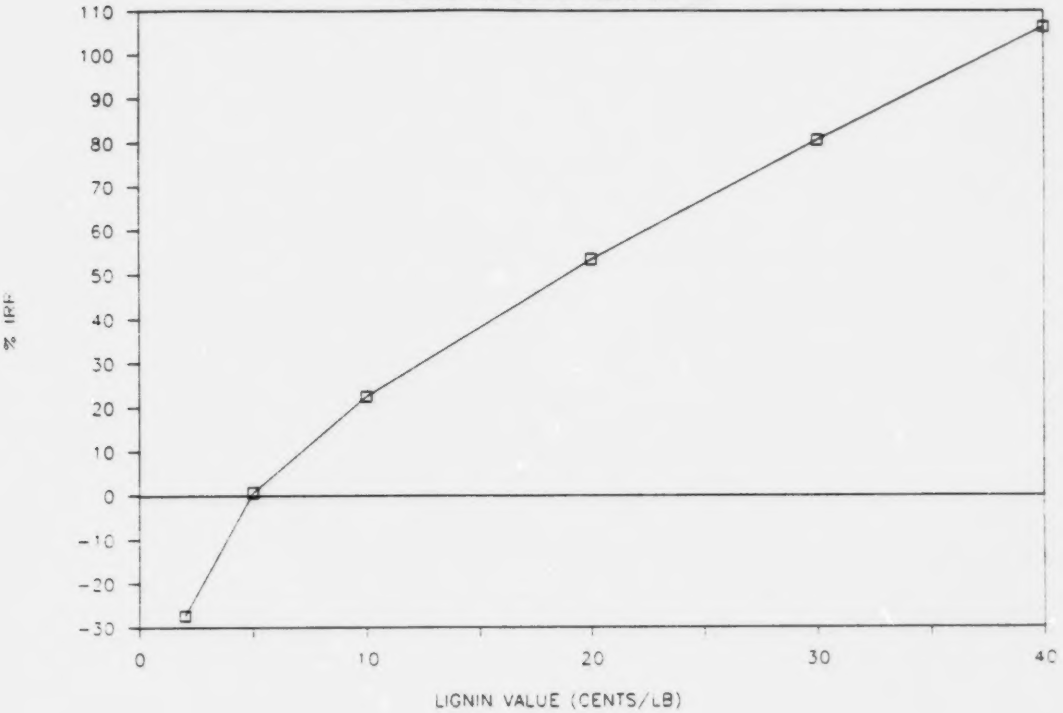


FIGURE NO. 7

EFFECT OF YIELD ON IRR

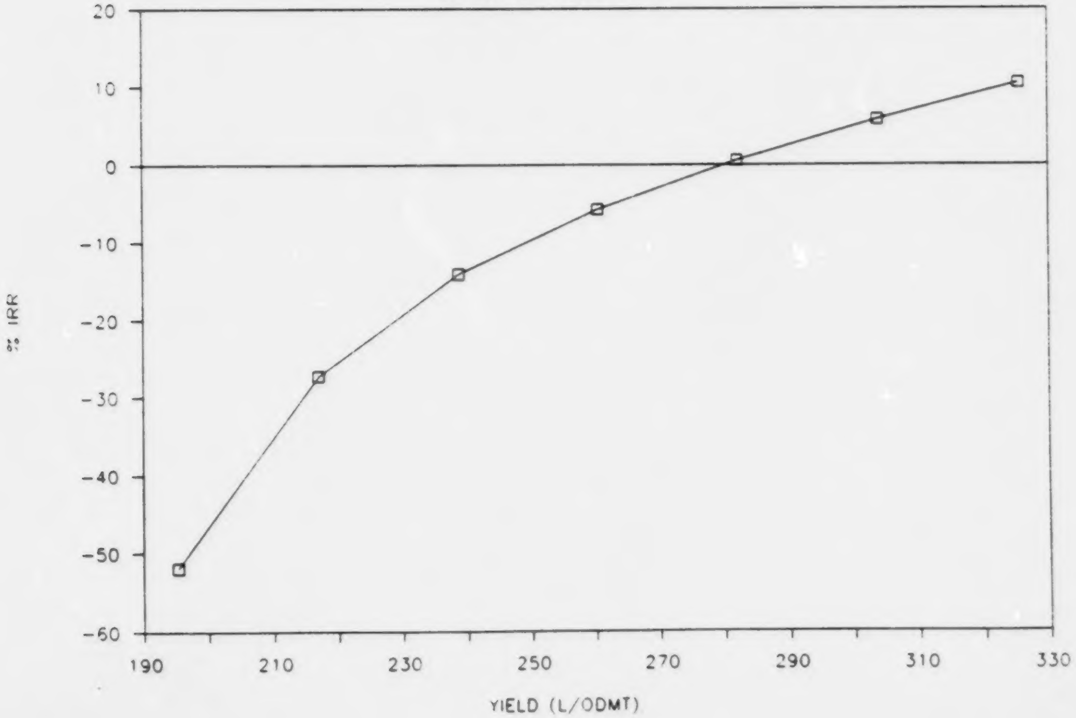


TABLE NO. 14

CASH FLOW PROJECTIONS UTILIZING A
10 CENTS/LBS VALUE FOR WOOD RESIDUE STREAM

PINE TO ETHANOL PLANT: 20,000,000 L/YR
January 1989

INTERNAL RATE OF RETURN: 22%
NET PRESENT VALUE (20%): $1,224,788

	YEAR 0	YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5	YEAR 6	YEAR 7	YEAR 8	YEAR 9	YEAR 10
INVESTMENT											
-LAND	$128,551										($177,693)
-BUILDINGS	$1,671,169										($1,000,591)
-PROC'G EQUIP(CLASS 29)	$12,855,145										($723,918)
-WORKING CAPITAL	$642,757										($642,757)
TOTAL INVESTMENT A	$15,297,623	$0	$0	$0	$0	$0	$0	$0	$0	$0	($2,544,960)
UNITS PRODUCED											
- ETHANOL (MILLION L)		15	20	20	20	20	20	20	20	20	20
- LIGNIN (MILLION LBS)		60.6	80.8	80.8	80.8	80.8	80.8	80.8	80.8	80.8	80.8
REVENUES											
- ETHANOL		$6,000,000	$8,320,000	$8,652,800	$8,998,912	$9,358,868	$9,733,223	$10,122,552	$10,527,454	$10,948,552	$11,386,494
- LIGNIN		$6,057,802	$8,400,152	$8,736,158	$9,085,604	$9,449,028	$9,826,989	$10,220,069	$10,628,872	$11,054,027	$11,496,188
TOTAL REVENUES	$0	$12,057,802	$16,720,152	$17,388,958	$18,084,516	$18,807,897	$19,560,213	$20,342,621	$21,156,326	$22,002,579	$22,882,682
VARIABLE COSTS											
- LABOUR		$720,000	$748,800	$778,752	$809,902	$842,298	$875,990	$911,030	$947,471	$985,370	$1,024,785
- FEEDSTOCK		$4,258,903	$4,429,259	$4,606,429	$4,790,687	$4,982,314	$5,181,607	$5,388,871	$5,604,426	$5,828,603	$6,061,747
- CHEMICALS		$810,507	$842,927	$876,644	$911,710	$948,179	$986,106	$1,025,550	$1,066,572	$1,109,235	$1,153,604
- PROCESS WATER		$153,338	$159,472	$165,850	$172,484	$179,384	$186,559	$194,021	$201,782	$209,854	$218,248
- ELECTRICITY		$633,641	$658,987	$685,346	$712,760	$741,270	$770,921	$801,758	$833,828	$867,181	$901,869
- STEAM		$3,248,854	$3,378,808	$3,513,960	$3,654,519	$3,800,700	$3,952,728	$4,110,837	$4,275,270	$4,446,281	$4,624,132
- MAINTENANCE		$555,342	$577,556	$600,658	$624,684	$649,672	$675,658	$702,685	$730,792	$760,024	$790,425
- SUPERVISORY		$108,000	$112,320	$116,813	$121,485	$126,345	$131,399	$136,654	$142,121	$147,805	$153,718
- ADMINISTRATION		$144,000	$149,760	$155,750	$161,980	$168,460	$175,198	$182,206	$189,494	$197,074	$204,957
TOTAL VAR. COSTS	$0	$10,632,585	$11,057,888	$11,500,204	$11,960,212	$12,438,621	$12,936,165	$13,453,612	$13,991,756	$14,551,427	$15,133,484
PRE-TAX CASH FLOWS	$0	$1,425,217	$5,662,263	$5,888,754	$6,124,304	$6,369,276	$6,624,047	$6,889,009	$7,164,570	$7,451,152	$7,749,198
TAX @ 40.8%	$0	$581,488	$2,310,203	$2,402,612	$2,498,716	$2,598,665	$2,702,611	$2,810,716	$2,923,144	$3,040,070	$3,161,673
INCOME AFTER TAX B	$0	$843,728	$3,352,060	$3,486,142	$3,625,588	$3,770,612	$3,921,436	$4,078,293	$4,241,425	$4,411,082	$4,587,525
TAX DEPRECIATION											
-BUILDINGS (5% d.b.)		$83,558	$79,381	$75,411	$71,641	$68,059	$64,656	$61,423	$58,352	$55,434	$52,663
-PROCESSING EQUIP (25% d.B.)		$3,213,786	$2,410,340	$1,807,755	$1,355,816	$1,016,862	$762,647	$571,985	$428,989	$321,742	$241,306
TOTAL CCA CLAIMED	$0	$3,297,345	$2,489,720	$1,883,166	$1,427,457	$1,084,921	$827,302	$633,408	$487,341	$377,176	$293,969
TAX RECOVERY C 40.8%	$0	$1,345,317	$1,015,806	$768,332	$582,402	$442,648	$337,539	$258,430	$198,835	$153,888	$119,939
NET CASH FLOW (-A+B+C)	($15,297,623)	$2,189,045	$4,367,866	$4,254,474	$4,207,990	$4,213,259	$4,258,975	$4,336,724	$4,440,260	$4,564,970	$7,252,424
ACCUMULATED	($15,297,623)	($13,108,578)	($8,740,712)	($4,486,238)	($278,247)	$3,935,012	$8,193,987	$12,530,711	$16,970,971	$21,535,941	$28,788,366

TABLE NO. 14 (CONTINUED)

VARIABLES REQUIRED PINE TO ETHANOL	INPUT VARIABLES	CALCULATED BY PROGRAM
OPERATIONAL CAPACITY YEAR 1	75%	
OPERATIONAL CAPACITY YEAR 2	100%	
ETHANOL PER YEAR (L)		20,000,000
ETHANOL ($/L)	$0.40	
LIGNIN PER YEAR (LBS)		80,770,690
LIGNIN ($/LBS)	$0.10	
E. OPERATING COSTS: ($/YR)		
FEEDSTOCK COST	$4,258,903	
INFLATION %	4.0%	
INVEST. TAX CREDIT(I.T.C.) RATE %	20.0%	
F. INVESTMENT: ($)		
PROCESSING EQUIPMENT (CLASS 29) INSTALLED & INSTR & CONT		12,855,145.0
TOTAL INDIRECT COSTS	15%	1,928,271.8
PIPING & ELECTRICAL	10%	1,285,514.5
TOTAL EQUIPMENT COST		16,068,931.3
ITC EQUIPMENT		(3,213,786.3)
INVESTMENT IN EQUIPMENT		12,855,145.0
LAND (% OF EQUIP)	1%	$128,551
BUILDING (% OF EQUIP)	13%	$1,671,169
WORKING CAPITAL (% OF EQUIP)	5.0%	$642,757
TOTAL FIXED CAPITAL INVESTMENT		$15,297,623
TAX RATE	40.8%	

ASSUMPTIONS USED IN MODEL

1. INVESTMENT ASSUMED ALL AT YEAR 0 AND NET OF ANY INVESTMENT TAX CREDIT
2. AT END OF TENTH YEAR INVESTED ITEMS ASSUMED DISPOSED OF AT FOLLOWING NET PROCEEDS:
    LAND - ASSUMED INCREASED IN VALUE EQUAL TO INFLATION AND GAIN TAXED AT HALF (CAP.GAIN)
    BUILDING AND EQUIPMENT - ASSUMED DISPOSED OF AT DEPRECIATED TAX VALUE (COST LESS ACC'D DEP'N)
    WORKING CAPITAL - ASSUMED ALL DISPOSED OF AT COST.
3. FOR TAX DEPRECIATION PURPOSES HALF YEAR RULE IGNORED (EXCEPT CLASS 29).
4. REVENUE AND COSTS PER UNIT ARE ASSUMED TO INCREASE AT INFLATION RATE.

7.0 CONCLUSIONS

1. The Wenger extruder was successful in performing the prehydrolysis of both pine and aspen, utilizing a feed containing up to 68 % D.S. and SO_2 as the catalyst. The maximum yields obtained from the extruder were approximately 24.7% (pine) and 22.9% (aspen), based on the C_5/C_6 sugars produced as a function of the wood feed. The temperature at the outlet of the extruder was approximately 170°C, while the $\%SO_2$/wood was about 1.5.

2. Washing of the extruded material in a two stage process incorporating a plate and frame filter press was able to accomplish approximately 95% sugar recovery.

3. Delignification was performed on the extruded material with both NaOH (autoclave, batch reactor and SLR reactor) and acetone (hot plate). The experiments with NaOH revealed that the aspen material was easier to delignify (50% removal from aspen versus 30% removal from pine), however high cellulose losses (over 50%) during processing of both woods, made delignification of the extruded wood with NaOH unattractive. The laboratory experiments performed with acetone resulted in the removal of 19% and 9% of the lignin from aspen and pine respectively.

4. Hydrolysis was accomplished in the SLR reactor with a feed of up to 17% D.S.. The maximum yields obtained for glucose based on the original wood feed, was 18.3% for the pine and 21.9% for the aspen. The maximum glucose yield was obtained from aspen at a maximum temperature of 226^{o}C and a residence time 1.4 minutes. The maximum glucose yield from pine was obtained at a maximum temperature of 220^{o}C and a residence time of 2.4 minutes.

5. Solid liquid separation of the hydrolysate was performed on both a decanter and centrifuge unit. Mass balances performed around the units demonstrated that 75% of the solubles could be recovered in a one stage process and approximately 95% of the solubles could be recovered in a two stage process.

6. Fermentation on the prehydrolysate, hydrolysate and mixed streams were performed primarily with Pichia stipitis. The fermentations were performed in vessels of up to 28-L. Steam stripping was performed on the prehydrolysate streams to remove the acetic acid before each fermentation. Fermentations of the prehydrolysate streams were carried out with initial sugar concentrations of up to 60-g/L. The yields obtained from the prehydrolysate streams were approximately 0.44 and 0.49-g ethanol/g sugar consumed for aspen and pine respectively with approximately 90% of the sugars consumed. Mixed (prehydrolysate/hydrolysate) streams

were fermented with initial sugar concentrations above 100-g/L. The yields obtained were 0.43 and 0.45 for aspen and pine respectively. The fermentation time and % sugars consumed were 66-hr and 96.6 for the aspen stream and 36-hr and 95.1 for the pine

7. An overall mass balance was developed for a 20,000,000-L/yr wood to ethanol plant integrated with a pulp and paper mill in northern Ontario. The feedstock considered, was the pine waste wood available that is burned in the plant to produce steam and electricity. The overall process yield of the plant was found to be 217-L/ODMT. This yield was lower than was projected in an earlier study (approximately 314-L/ODMT) due to the consideration of washing efficiencies, a lower yield obtained with the extruder than was obtained with the plug flow reactor for prehydrolysis and the fact that the recycle of the processed material into the plug flow reactor was found to be uneconomical. The estimated capital cost of the plant was $18,500,000.

8. Based on a raw material cost for the plant of $46.20/ODMT and an equivalent value for the lignin rich solid residue produced, an IRR of -27.22% was calculated. A sensitivity analysis performed revealed that the value of the lignin rich residue has the most significant effect on the IRR of the plant. It was determined that an IRR of 22% could be achieved utilizing the present feedstock cost, if

the residue was valued at $0.10/lbs. It is uncertain if the solid residue which is rich in lignin (>60%) could be sold for $0.10/lbs. A study is presently being conducted at Bio-Hol to determine what is the best way to deal with the residue material.

8.0 FUTURE WORK AND RECOMMENDATIONS

1. The analysis performed in this project demonstrated the importance of the value of the lignin rich residue stream to the overall economics of the wood to ethanol plant. It is therefore important that work continue as is being done presently at Bio-Hol, for evaluating the residue and determining methods to up-grade its value.

2. It was noted during this study that the recycling of the hydrolyzed material back into the plug flow reactor was uneconomical due to the low cellulose content of the material. It is believed that the successful isolation of the cellulose fraction from the hydrolyzed material would render recycling economical. Research into the successful isolation of the cellulose fraction of the hydrolyzed material is also being performed presently at Bio-Hol.

3. The fermentations performed in this project utilized YMP (yeast; malt; peptone) as the nutrient. It is recommended that further studies be done quantifying the use of less expensive nutrients (ie. corn steep liquor).

9.0 REFERENCES

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3. Laura Burns, Dorr Oliver, personal communication.

4. Lars Firing, Firing Industries, personal communication.

5. Steve Levin, GEA-Wiegand Division, personal communication.

6. Brian Smith, TCI Superior, personal communication.

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APPENDIX I

EXTRUDER SYSTEM EQUIPMENT LIST

WENGER SYSTEM EQUIPMENT LIST

Component	Manufacturer	Description
Extruder	Wenger, SX80	Barrel and Screw
Hydraulic Motor	U.S. Electrical Motors	7.5 H.P.
Temperature Probes		Type "T" Thermocouples
Pressure Transducers	Gentran Inc.	Three, (0-3000 psi)
Pressure Gauges	LHA	Three, (0-5000 psi)
Live Bin (Extruder)	Wenger Mnfg.	Hydraulic Motor, (capacity =
Cyclone	Torit	
Vibrator	Syntron (Model BF2A)	
Ribbon Blender		(capacity = 1/2 Ton)
Feeder System (Extruder)		
Recorder	YEW (Model 3088)	30 Channel
Acid Pump	Milton Roy	Capacity = 300 cm^3/min.
Water Pump	Milton Roy	Capacity = 300 cm^3/min.

APPENDIX II

METHODS OF ANALYSIS

METHODS OF ANALYSIS

1.0 Dry Solids

Duplicates of the well mixed sample were weighed out in tared crucibles, then transferred to a convection oven at 80°C for 24 hours. After cooling the crucibles were weighed and the % D.S. calculated as follows:

$$\frac{\text{wt in g of dried sample}}{\text{wt in g of test specimen}} \times 100 = \%\text{D.S.}$$

2.0 Extractives

The method used was Standard G.20 approved by the Canadian Pulp and Paper Association, Physical and Chemical Standards Committee, Technical Section, with one exception. Due to the hazards of benzene use, hexane was substituted.

3.0 Cellulose, Hemicellulose and Acid Insoluble (Klason) Lignin

To determine cellulose and hemicellulose, a procedure was employed that allows determination simultaneously with acid insoluble lignin.

One gram of dry, soluble free material (< 28 mesh) was carefully weighed and placed in a 100-mL beaker. All samples were performed in duplicate. 15-mL of 72% H_2SO_4 at approximately 10°C was added and mixed with the material. The beaker was placed in a water bath at 20°C for 2 hours and mixed occasionally. After 2 hours, the mixture was transferred quantitatively to a 500-mL Erlynmyer flask and diluted to a final volume of 415-mL. The final concentration of H_2SO_4 is now 4% w/w.

Next, the flasks were autoclaved for 2 hours at 15-psig and 121°C. At this point hydrolysis of the cellulose and hemicellulose in the material should be completed with only sugar monomers present. The solution was then cooled to room temperature and its volume noted. The solution was filtered quantitatively through a weigh Whatman GF/A filter paper, and the collected residue was dried in a convection oven (<70°C). This residue is classified as acid insoluble (Klason) lignin. It is calculated as follows:

$$\frac{\text{wt. in g of dry residue}}{\text{wt. in g of test specimen}} \times 100 = \%\ \text{Lignin}$$

The filtrate now contains the monomers of both the hemicellulose and cellulose, and before injection into the HPLC, it must be neutralized and deashed. One hundred mL of filtrate was treated with 4.5-g of $CaCO_3$. The H_2SO_4 reacts according to:

$$CaCO_3 + H_2SO_4 \quad > \quad CaSO_4 + H_2CO_3 + CO_2$$

Filtering gives a solution free of ash. The solution was filtered through a 0.45-uM Millipore HPLC Sample filter and injected into the HPLC. A tracing is obtained under the following conditions:

Injection volume = 100-uL
Column temperature = 40^oC
Mobile phase = 0.01-N H_2SO_4
Flowrate = 0.6-mL/min

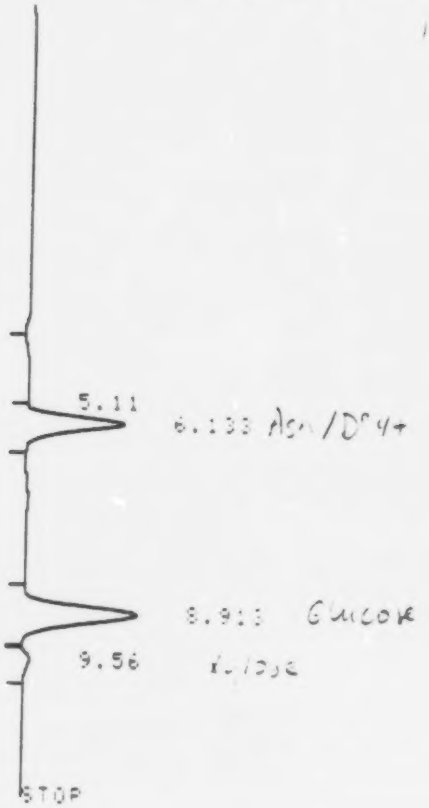
equipment: Bio-Rad HPX 87H column
Waters R-401 Differential Refractometer
Beckman 110 B Solvent Delivery Module
Shimadzu C-R3A Chromatopac Integrator

From the tracing the percentages of cellulose and hemicellulose are determined using appropriate calculations, taking into account volume changes and chemical gain. A sample HPLC tracing follows as does a sample calculation. It is important to note that it is assumed that all dimers, trimers and highers are oligomers of glucose. The mannose, xylose and galactose peaks are not always separable.

```
PRINT LEVEL
 43.6667
A.SAVE
START
```

SJ-5 Aspen Tr hydrolyses

100uL



```
STOP

CHROMATOGRAM   1  MEMORIZED

CHROMATOPAC   C-R3A                          FILE          3
SAMPLE NO    0                               METHOD          64
REPORT NO    621                             SAMPLE WT     100

PKNO       TIME        AREA  MK  IDNO       CONC        NAME

   1       5.11        17805
   2       6.133      102395  V                             5/L
   3       8.913      136592         3         0.1539 GLU   1.80
   4       9.56         8357         4         0.0136 XYL u 0.11
                   ---------           --------------
         TOTAL        265149                   0.1675
```

Sample calculation:

Dry weight of material = 1.0008-g
Volume after autoclave = 400-mL

8.913 peak (glucose) - conc = 1.80-g/L
= 0.18-g in 100-mL
in 400-mL = 0.72-g

% Glucose = 0.72/1.008 x 100
= 71.94%

Chemical Gain = 71.94 - 0.1 x 71.94
= 64.7%

9.56 peak (xylose) - conc = 0.11-g/L
% Xylose = 0.11/1.80 x 100
= 4.40%

Chemical Gain = 4.40 - 0.12 x 4.40
= 3.9%

Cellulose = 64.7% *
Hemicellulose = 3.9%

* This is the percentage of cellulose assuming no starch was present in the original material. If starch was present (as determined independently using a standard starch analysis) it is simply subtracted from the cellulose figure to give a true cellulose content.

4.0 Soluble and Particulate Fractions of Prehydrolysis and Hydrolysis Products

This method is used to determine the soluble insoluble fractions of these products.

A well mixed sample of the as is product was weighed (about 100-g) and slurried with an excess of hot water. The slurry was quantitatively filtered through a weighed filter paper and washed with hot water till the filtrate is soluble free (approximately 500-mL hot water). Both the residue and filtrate were oven dried. It was assumed that all the solubles in the product were contained in the filtrate and there was no loss of volatile material. The soluble/insoluble split is calculated as follows:

$$\frac{\text{wt in g of oven dried filtrate}}{(\text{wt in g of oven dried filtrate} + \text{wt in g of oven dried residue})} \times 100 = \%\ \text{Solubles}$$

$$\frac{\text{wt in g of oven dried residue}}{(\text{wt in g of oven dried residues} + \text{wt in g of oven dried filtrate})} \times 100 = \%\ \text{Insolubles}$$

5.0 Identification of Solubles in Prehydrolysis and Hydrolysis Products

The HPLC is used to determine the nature of the soluble fractions of these products.

Depending on the properties of the products, a water addition step may be necessary prior to HPLC injection. If a free liquid phase cannot be easily generated, add sufficient water to produce this phase. The slurry is neutralized with NaOH and centrifuged. The filtrate was decanted and as aforementioned, prepared for injection. The tracing was used to determine the total detectable sugars and degradable products of the sample. Sample tracings of both prehydrolysis and hydrolysis samples are included.

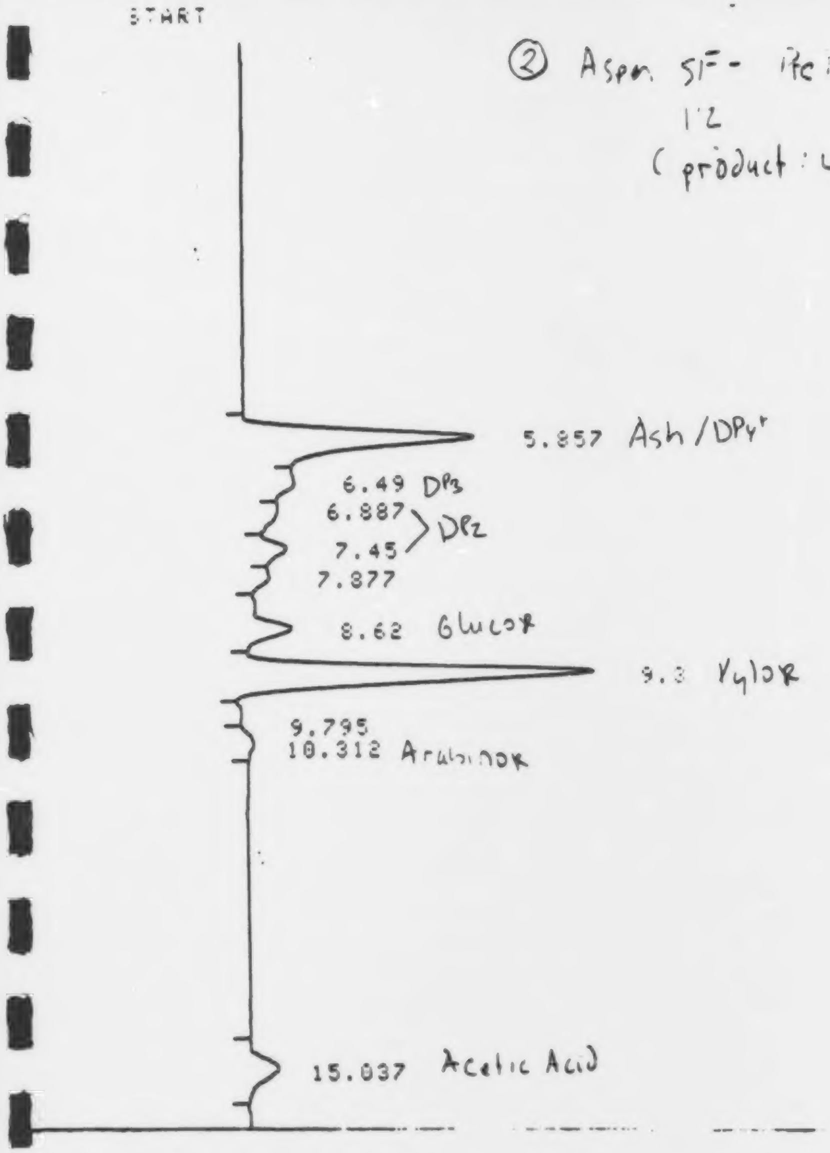
6.0 % SO_2 Determination

SO_2 was used as the catalyst in the prehydrolysis step. An accurate determination was achieved by using an oxidation with Iodine in acid environment method. The SO_2 was in the form of H_2SO_3, sulfurous acid, produced by bubbling gaseous SO_2 through water.

The sulfurous acid was accurately diluted 1:9 with water in a 100-mL volumetric flask. Then 30-40-mL 0.1-N Iodine and 10-mL of 10% HCl were accurately measured into a 250-mL Erlynmeyer flask. The flask was placed on a zeroed balance, and the sample added by pipet, making sure the tip of the pipet was below the surface of the Iodine solution to prevent air oxidation. If at this point the solution turns colorless, a larger volume of Iodine solution is required. The weight of the added sulfurous acid was noted. Titrate with 0.1-N thiosulfate until yellow, add 1-mL starch indicator and continue till colorless:

$$\frac{(\ (I_2\text{-mL} - Na_2SO_4\text{-mL} \times I_2\text{-N}/Na_2SO_4\text{-N}) \times I_2\text{-N} \times 0.016032 \times 100)}{\text{wt in grams of solution analyzer}} = \%S$$

② Aspen SF- Prehydrolysis
1:2
(product : water , w/w)



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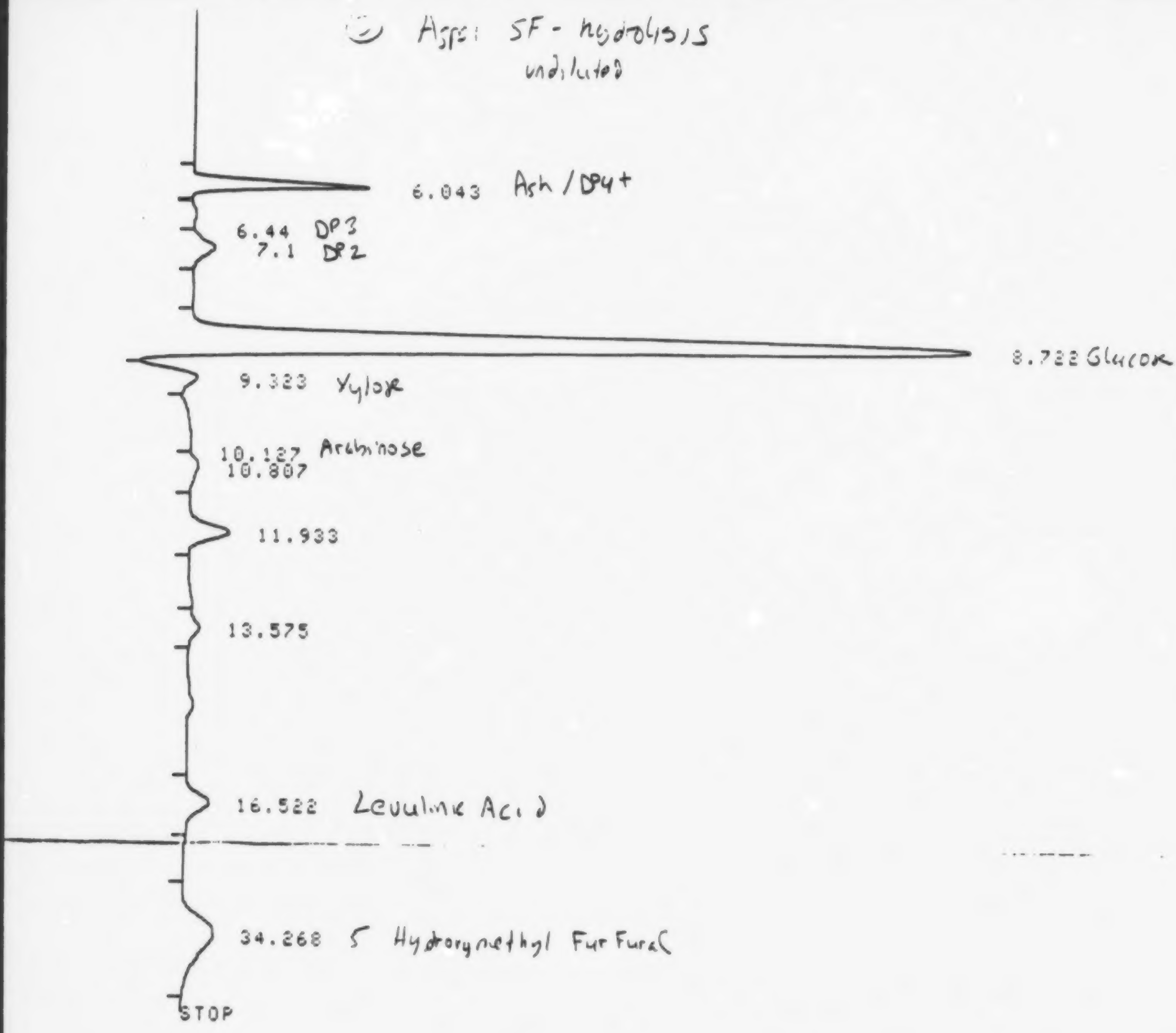
CHROMATOPAC   C-R3A                        FILE          3
SAMPLE NO   0                              METHOD          64
REPORT NO   184                            SAMPLE WT    100

PKNO      TIME       AREA   MK   IDNO       CONC          NAME

                                                                    X3
   1      5.857     321863
   2      6.49      100734   V     1          0.192  MTR    2.81    8.43
   3      6.887      64532   V     2          0.1143 MALT
                                                         > 6.10.  18.3
   4      7.45       71459   V     2          0.1305 MALT
   5      7.877      42608   V
   6      8.62       91934   V     3          0.1082 GLU     5.30   15.9
   7      9.3       406924   V     4          0.505  XYL    24.93   74.79
   8      9.795       4303         4          0.0103 XYL
   9     10.312      13178   V     5          0.0261 ARAB    1.27    3.81
  10     15.037      47611         8          0.124  ACETIC  6.05   18.15
                 ---------                 ------------
          TOTAL    1165144                    1.2104
```



Asps: SF - hydrolysis
undiluted

```
CHROMATOPAC    C-R3A
SAMPLE NO   0                              FILE          3
REPORT NO    518                           METHOD          64
                                           SAMPLE WT    100

PKNO        TIME        AREA   MK   IDNO        CONC         NAME
                                                                  g/L
   1       6.043      123797
   2       6.44         8210          1       -0.0382  MTR     7.17
   3       7.1         36103   V      2        0.0275  MALT    1.61
   4       8.722     1182884          3        1.2992  GLU    63.60
   5       9.323       98277          4        0.1204  XYL     6.14
   6      10.127      155691   V      5        0.125   ARAB    6.20
   7      10.807       92728   V
   8      11.933      107651   V      6        0.2718  LACTIC
   9      13.575       13210
  10      16.522       43109
  11      34.268      111818                          Levulinic Acid 340.
                  ----------           --------------   5 HMF         625
          TOTAL      1973476                   1.8057
```

APPENDIX III

MASS BALANCES EXTRUDER RUNS

MASS BALANCE: WP-1

FLOWRATES: LBS/MIN

ASSUME A CHEMICAL GAIN BASED ON 100%
CONVERSION OF HEMICELLULOSE

	WOOD FEED	SO2 FEED	PRODUCT	FLASH
FLOW	4.24	0.79	4.23	0.80
DRY SOLIDS	2.34	0.04	2.43	
HEMICELLULOSE	0.44	0.00	0.00	
CELLULOSE	1.05	0.00	0.63	
LIGNIN	0.74	0.00	0.88	
WATER	1.90	0.75	1.80	
SO2	0.00	0.04	0.04	
GLUCOSE	0.00	0.00	0.13	
XYLOSE	0.00	0.00	0.37	
ARABINOSE	0.00	0.00	0.03	
LACTIC ACID	0.00	0.00	0.04	
ACETIC ACID	0.00	0.00	0.00	
%DRY SOLIDS	55.20	5.20	57.50	
%SOLUBLES	0.00	100.00	32.00	
%INSOLUBLES	100.00	0.00	68.00	

CONVERSIONS:

XYLOSE/HC = (0.37/(1.136*0.44))*100 = 72.3%

XYLOSE/DRY WOOD FEED = 0.37/2.34 * 100 = 15.6%

GLUCOSE/DRY WOOD FEED = 0.13/2.34 * 100 = 5.41%

ARABINOSE/DRY WOOD = 0.03/2.34 * 100 = 1.25%

LACTIC ACID/DRY WOOD = 0.04/2.34 * 100 = 1.57%

CELLULOSE = (1-0.63/1.05) * 100 =39.24%

MASS BALANCE: WP-2

FLOWRATES: LBS/MIN

ASSUME A CHEMICAL GAIN BASED ON 100%
CONVERSION OF HEMICELLULOSE

	WOOD FEED	SO2 FEED	PRODUCT	FLASH
FLOW	5.24	0.88	5.00	1.12
DRY SOLIDS	2.88	0.03	3.01	
HEMICELLULOSE	0.55	0.00	0.00	
CELLULOSE	1.29	0.00	1.00	
LIGNIN	0.91	0.00	0.88	
WATER	2.36	0.85	1.99	
SO2	0.00	0.03	0.03	
GLUCOSE	0.00	0.00	0.17	
XYLOSE	0.00	0.00	0.50	
ARABINOSE	0.00	0.00	0.05	
LACTIC ACID	0.00	0.00	0.04	
ACETIC ACID	0.00	0.00	0.04	
%DRY SOLIDS	55.00	3.60	60.20	
%SOLUBLES	0.00	100.00	34.70	
%INSOLUBLES	100.00	0.00	65.30	

CONVERSIONS:

XYLOSE/HC = (0.50/(1.136*0.55))*100 = 80.20%

XYLOSE/DRY WOOD FEED = 0.50/2.88 * 100 = 17.31%

GLUCOSE/DRY WOOD FEED = 0.17/2.88 * 100 = 5.75%

ARABINOSE/DRY WOOD = 0.05/2.88 * 100 = 1.59%

LACTIC ACID/DRY WOOD = 0.04/2.88 * 100 = 1.30%

ACETIC ACID/WOOD = 0.04/2.88 *100 = 1.23%

CELLULOSE = (1-1.00/1.29) * 100 =22.68%

MASS BALANCE: WP-3

FLOWRATES: LBS/MIN

ASSUME A CHEMICAL GAIN BASED ON 100%
CONVERSION OF HEMICELLULOSE

	WOOD FEED	SO2 FEED	PRODUCT	FLASH
FLOW	8.73	1.32	8.40	1.65
DRY SOLIDS	5.15	0.07	5.36	
HEMICELLULOSE	0.98	0.00	0.00	
CELLULOSE	2.31	0.00	1.85	
LIGNIN	1.63	0.00	1.57	
WATER	3.58	1.25	3.04	
SO2	0.00	0.07	0.07	
GLUCOSE	0.00	0.00	0.21	
XYLOSE	0.00	0.00	0.90	
ARABINOSE	0.00	0.00	0.07	
LACTIC ACID	0.00	0.00	0.00	
ACETIC ACID	0.00	0.00	0.07	
%DRY SOLIDS	59.00	5.30	63.80	
%SOLUBLES	0.00	100.00	33.10	
%INSOLUBLES	100.00	0.00	66.90	

CONVERSIONS:

XYLOSE/HC = (0.90/(1.136*0.98))*100 = 81.40%

XYLOSE/DRY WOOD FEED = 0.90/5.15 * 100 = 17.56%

GLUCOSE/DRY WOOD FEED = 0.21/5.15 * 100 = 4.03%

ARABINOSE/DRY WOOD = 0.07/5.15 * 100 = 1.34%

LACTIC ACID/DRY WOOD = 0.00

ACETIC ACID/WOOD = 0.07/5.15 *100 = 1.34%

CELLULOSE = (1-1.85/2.31) * 100 =20.10%

MASS BALANCE: WA-1

FLOWRATES: LBS/MIN

ASSUME A CHEMICAL GAIN BASED ON 100%
CONVERSION OF HEMICELLULOSE

	WOOD FEED	SO2 FEED	PRODUCT	FLASH
FLOW	2.42	1.28	3.15	0.55
DRY SOLIDS	1.65	0.05	1.73	
HEMICELLULOSE	0.30	0.00	0.00	
CELLULOSE	0.83	0.00	0.70	
LIGNIN	0.40	0.00	0.33	
WATER	0.77	1.23	1.42	
SO2	0.00	0.05	0.05	
GLUCOSE	0.00	0.00	0.07	
XYLOSE	0.00	0.00	0.24	
ARABINOSE	0.00	0.00	0.00	
LACTIC ACID	0.00	0.00	0.00	
ACETIC ACID	0.00	0.00	0.03	
%DRY SOLIDS	68.10	3.60	54.90	
%SOLUBLES	0.00	100.00	38.80	
%INSOLUBLES	100.00	0.00	61.20	

CONVERSIONS:

XYLOSE/HC = (0.24/(1.136*0.30))*100 = 70.42%

XYLOSE/DRY WOOD FEED = 0.24/1.65 * 100 = 14.48%

GLUCOSE/DRY WOOD FEED = 0.07/1.65 * 100 = 4.44%

ARABINOSE/DRY WOOD = 0.00

LACTIC ACID/DRY WOOD = 0.00

ACETIC ACID/WOOD = 0.03/1.65 *100 = 1.77%

CELLULOSE = (1-0.70/0.83) * 100 = 16.20%

MASS BALANCE: WA-2

FLOWRATES: LBS/MIN

ASSUME A CHEMICAL GAIN BASED ON 100% CONVERSION OF HEMICELLULOSE

	WOOD FEED	SO2 FEED	PRODUCT	FLASH
FLOW	3.95	1.28	4.50	0.73
DRY SOLIDS	2.24	0.03	2.34	
HEMICELLULOSE	0.41	0.00	0.00	
CELLULOSE	1.13	0.00	1.20	
LIGNIN	0.54	0.00	0.92	
WATER	1.71	1.25	2.16	
SO2	0.00	0.03	0.03	
GLUCOSE	0.00	0.00	0.09	
XYLOSE	0.00	0.00	0.35	
ARABINOSE	0.00	0.00	0.00	
LACTIC ACID	0.00	0.00	0.00	
ACETIC ACID	0.00	0.00	0.07	
%DRY SOLIDS	56.60	2.40	52.10	
%SOLUBLES	0.00	100.00	37.00	
%INSOLUBLES	100.00	0.00	63.00	

CONVERSIONS:

XYLOSE/HC = (0.35/(1.136*0.41))*100 = 76.80%

XYLOSE/DRY WOOD FEED = 0.35/2.24 * 100 = 15.80%

GLUCOSE/DRY WOOD FEED = 0.09/2.24 * 100 = 3.80%

ARABINOSE/DRY WOOD = 0.00

LACTIC ACID/DRY WOOD = 0.00

ACETIC ACID/WOOD = 0.07/2.24 *100 = 3.25%

CELLULOSE = (1-0.75/1.13) * 100 = 33.20%

MASS BALANCE: WA-3

FLOWRATES: LBS/MIN

ASSUME A CHEMICAL GAIN BASED ON 100%
CONVERSION OF HEMICELLULOSE

	WOOD FEED	SO2 FEED	PRODUCT	FLASH
FLOW	3.76	1.26	4.21	0.81
DRY SOLIDS	2.21	0.04	2.30	
HEMICELLULOSE	0.40	0.00	0.00	
CELLULOSE	1.12	0.00	0.94	
LIGNIN	0.53	0.00	0.45	
WATER	1.55	1.22	1.91	
SO2	0.00	0.04	0.04	
GLUCOSE	0.00	0.00	0.05	
XYLOSE	0.00	0.00	0.26	
ARABINOSE	0.00	0.00	0.01	
LACTIC ACID	0.00	0.00	0.00	
ACETIC ACID	0.00	0.00	0.06	
%DRY SOLIDS	58.70	2.90	54.60	
%SOLUBLES	0.00	100.00	32.50	
%INSOLUBLES	100.00	0.00	67.50	

CONVERSIONS:

XYLOSE/HC = (0.26/(1.136*0.40))*100 = 65.98%

XYLOSE/DRY WOOD FEED = 0.26/2.21 * 100 = 11.94%

GLUCOSE/DRY WOOD FEED = 0.05/2.21 * 100 = 2.34%

ARABINOSE/DRY WOOD = 0.01/2.21 *100 = 0.60%

LACTIC ACID/DRY WOOD = 0.00

ACETIC ACID/WOOD = 0.06/2.21 *100 = 2.57%

CELLULOSE = (1-0.94/1.12) * 100 = 15.96%

MASS BALANCE: WA-4

FLOWRATES: LBS/MIN

ASSUME A CHEMICAL GAIN BASED ON 100%
CONVERSION OF HEMICELLULOSE

	WOOD FEED	SO2 FEED	PRODUCT	FLASH
FLOW	5.95	1.19	6.20	0.94
DRY SOLIDS	4.00	0.06	4.17	
HEMICELLULOSE	0.72	0.00	0.00	
CELLULOSE	2.02	0.00	1.71	
LIGNIN	0.96	0.00	0.84	
WATER	1.95	1.13	2.03	
SO2	0.00	0.06	0.06	
GLUCOSE	0.00	0.00	0.13	
XYLOSE	0.00	0.00	0.67	
ARABINOSE	0.00	0.00	0.02	
LACTIC ACID	0.00	0.00	0.00	
ACETIC ACID	0.00	0.00	0.13	
%DRY SOLIDS	67.20	4.90	67.30	
%SOLUBLES	0.00	100.00	33.80	
%INSOLUBLES	100.00	0.00	66.20	

CONVERSIONS:

XYLOSE/HC = (0.67/(1.136*0.72))*100 = 81.26%

XYLOSE/DRY WOOD FEED = 0.67/4.00 * 100 = 16.71%

GLUCOSE/DRY WOOD FEED = 0.13/4.00 * 100 = 3.31%

ARABINOSE/DRY WOOD = 0.02/4.00 *100 = 0.38%

LACTIC ACID/DRY WOOD = 0.00

ACETIC ACID/WOOD = 0.13/4.00 *100 = 3.26%

CELLULOSE = (1-1.71/2.02) * 100 = 15.52%

MASS BALANCE: WA-5

FLOWRATES: LBS/MIN

ASSUME A CHEMICAL GAIN BASED ON 100%
CONVERSION OF HEMICELLULOSE

	WOOD FEED	SO2 FEED	PRODUCT	FLASH
FLOW	6.25	1.06	6.10	1.21
DRY SOLIDS	4.05	0.06	4.22	
HEMICELLULOSE	0.73	0.00	0.00	
CELLULOSE	2.05	0.00	1.88	
LIGNIN	0.98	0.00	0.89	
WATER	2.20	1.00	1.88	
SO2	0.00	0.06	0.06	
GLUCOSE	0.00	0.00	0.16	
XYLOSE	0.00	0.00	0.57	
ARABINOSE	0.00	0.00	0.01	
LACTIC ACID	0.00	0.00	0.03	
ACETIC ACID	0.00	0.00	0.11	
%DRY SOLIDS	64.80	5.80	69.20	
%SOLUBLES	0.00	100.00	34.20	
%INSOLUBLES	100.00	0.00	65.80	

CONVERSIONS:

XYLOSE/HC = (0.57/(1.136*0.73))*100 = 68.48%

XYLOSE/DRY WOOD FEED = 0.57/4.05 * 100 = 14.08%

GLUCOSE/DRY WOOD FEED = 0.16/4.05 * 100 = 3.93%

ARABINOSE/DRY WOOD = 0.01/4.05 *100 = 0.26%

LACTIC ACID/DRY WOOD = 0.03/4.05 * 100 = 0.78%

ACETIC ACID/WOOD = 0.11/4.05 *100 = 2.68%

CELLULOSE = (1-1.88/2.05) * 100 = 8.45%

APPENDIX IV

MASS BALANCES PLUG FLOW RUNS

MASS BALANCE PH-1

FEED:	D.S. %	14.53
	pH	1.5
	ACID STRENGTH %	0.28
	ACID/WOOD %	1.64
	HCL (LBS)	1.19
	WATER (LBS)	429.99
	DRY SOLIDS (LBS)	72.65
	CELLULOSE (LBS)	27.97

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	220
RESIDENCE TIME (MIN)	1.58
% FLASH CALCULATED	20.65

PRODUCTS:

% D.S.	17.21
WATER (LBS)	364.93
DRY SOLIDS (LBS)	75.86
SOLUBLES (LBS)	24.38
INSOLUBLES (LBS)	51.48
CELLULOSE (LBS)	14.31
LIGNIN (LBS)	34.54
GLUCOSE (LBS)	17.87
LEVULINIC ACID (LBS)	1.15
5-HMF (LBS)	1.34

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	17.87/(72.65*0.385*1.111)
	57.5
GLUCOSE/WOOD FEED %	24.6
CELLULOSE CONVERSION %	49.6

MASS BALANCE PH-2

FEED:	D.S. %	17.2
	pH	1.5
	ACID STRENGTH %	0.3
	ACID/WOOD %	1.5
	HCL (LBS)	0.75
	WATER (LBS)	250.1
	DRY SOLIDS (LBS)	51.6
	CELLULOSE (LBS)	19.6

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	216
RESIDENCE TIME (MIN)	1.4
% FLASH CALCULATED	19.66

PRODUCTS:

% D.S.	19.3
WATER (LBS)	224.88
DRY SOLIDS (LBS)	53.78
SOLUBLES (LBS)	17.49
INSOLUBLES (LBS)	36.29
CELLULOSE (LBS)	6.53
LIGNIN (LBS)	28.42
GLUCOSE (LBS)	12.66
LEVULINIC ACID (LBS)	0.74
5-HMF (LBS)	0.92

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	58.11
GLUCOSE/WOOD FEED %	24.53
CELLULOSE CONVERSION %	68

MASS BALANCE PH-3

FEED:	D.S. %	17.5
	pH	1.5
	ACID STRENGTH %	0.32
	ACID/WOOD %	1.5
	HCL (LBS)	0.79
	WATER (LBS)	249.26
	DRY SOLIDS (LBS)	52.5
	CELLULOSE (LBS)	19.95

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	220
RESIDENCE TIME (MIN)	2.4
% FLASH CALCULATED	20.29

PRODUCTS:

% D.S.	19.1
WATER (LBS)	198.69
DRY SOLIDS (LBS)	54.75
SOLUBLES (LBS)	21.82
INSOLUBLES (LBS)	32.93
CELLULOSE (LBS)	6.49
LIGNIN (LBS)	25.03
GLUCOSE (LBS)	14.41
LEVULINIC ACID (LBS)	2.14
5-HMF (LBS)	1.71

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	65.03
GLUCOSE/WOOD FEED %	27.45
CELLULOSE CONVERSION %	68.8

MASS BALANCE PH-4

FEED:	D.S. %	14.6
	pH	1.6
	ACID STRENGTH %	0.3
	ACID/WOOD %	1.8
	HCL (LBS)	0.79
	WATER (LBS)	263.1
	DRY SOLIDS (LBS)	44.7
	CELLULOSE (LBS)	22.75

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	223
RESIDENCE TIME (MIN)	1.5
% FLASH CALCULATED	21.1

PRODUCTS:

% D.S.	18.1
WATER (LBS)	211.45
DRY SOLIDS (LBS)	45.94
SOLUBLES (LBS)	17.38
INSOLUBLES (LBS)	29.35
CELLULOSE (LBS)	5.19
LIGNIN (LBS)	22.78
GLUCOSE (LBS)	12.55
LEVULINIC ACID (LBS)	1.45
5-HMF (LBS)	1.45

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	49.65
GLUCOSE/WOOD FEED %	28.08
CELLULOSE CONVERSION %	78.2

MASS BALANCE PH-5

FEED:	D.S. %	15.9
	pH	1.6
	ACID STRENGTH %	0.31
	ACID/WOOD %	1.6
	HCL (LBS)	0.95
	WATER (LBS)	306.3
	DRY SOLIDS (LBS)	57.9
	CELLULOSE (LBS)	29.47

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	223
RESIDENCE TIME (MIN)	2.1
% FLASH CALCULATED	21

PRODUCTS:

% D.S.	19.2
WATER (LBS)	254.4
DRY SOLIDS (LBS)	59.5
SOLUBLES (LBS)	20.17
INSOLUBLES (LBS)	39.33
CELLULOSE (LBS)	8.26
LIGNIN (LBS)	29.77
GLUCOSE (LBS)	14.98
LEVULINIC ACID (LBS)	1.93
5-HMF (LBS)	1.73
LACTIC ACID (LBS)	1.17
GLYCEROL (LBS)	0.42

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	45.8
GLUCOSE/WOOD FEED %	25.87
CELLULOSE CONVERSION %	72.7

MASS BALANCE PH-6

FEED:	D.S. %	17.2
	pH	1.6
	ACID STRENGTH %	0.3
	ACID/WOOD %	1.5
	HCL (LBS)	0.74
	WATER (LBS)	240.7
	DRY SOLIDS (LBS)	50
	CELLULOSE (LBS)	25.7

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	223
RESIDENCE TIME (MIN)	1.68
% FLASH CALCULATED	20.86

PRODUCTS:

% D.S.	19.7
WATER (LBS)	212.47
DRY SOLIDS (LBS)	51.39
SOLUBLES (LBS)	21
INSOLUBLES (LBS)	31.12
CELLULOSE (LBS)	6.88
LIGNIN (LBS)	22.62
GLUCOSE (LBS)	13.59
LEVULINIC ACID (LBS)	1.3
5-HMF (LBS)	1.3
LACTIC ACID (LBS)	1.7

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	47.6
GLUCOSE/WOOD FEED %	27.2
CELLULOSE CONVERSION %	73.2

MASS BALANCE AH-1

FEED:	D.S. %	15.57
	pH	1.5
	ACID STRENGTH %	0.27
	ACID/WOOD %	1.48
	HCL (LBS)	0.79
	WATER (LBS)	291.35
	DRY SOLIDS (LBS)	53.41
	CELLULOSE (LBS)	35.3

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	220
RESIDENCE TIME (MIN)	2.27
% FLASH CALCULATED	20.52

PRODUCTS:

% D.S.	17
WATER (LBS)	267.2
DRY SOLIDS (LBS)	54.89
SOLUBLES (LBS)	22.49
INSOLUBLES (LBS)	33.19
CELLULOSE (LBS)	12.68
LIGNIN (LBS)	17.79
GLUCOSE (LBS)	17.34
LEVULINIC ACID (LBS)	0.91
5-HMF (LBS)	1.19
LACTIC ACID (LBS)	3.12

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	44.2
GLUCOSE/WOOD FEED %	32.47
CELLULOSE CONVERSION %	64.1

MASS BALANCE AH-2

FEED:	D.S. %	17.4
	pH	1.55
	ACID STRENGTH %	0.3
	ACID/WOOD %	1.5
	HCL (LBS)	0.89
	WATER (LBS)	291.08
	DRY SOLIDS (LBS)	60.9
	CELLULOSE (LBS)	36.84

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	219
RESIDENCE TIME (MIN)	1.38
% FLASH CALCULATED	20.17

PRODUCTS:

% D.S.	19.1
WATER (LBS)	264.2
DRY SOLIDS (LBS)	62.59
SOLUBLES (LBS)	18.96
INSOLUBLES (LBS)	43.63
CELLULOSE (LBS)	22.08
LIGNIN (LBS)	17.06
GLUCOSE (LBS)	13.85
LEVULINIC ACID (LBS)	0
5-HMF (LBS)	0.74
LACTIC ACID (LBS)	0.74
ACETIC ACID (LBS)	0.57

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	33.8
GLUCOSE/WOOD FEED %	22.74
CELLULOSE CONVERSION %	40.1

MASS BALANCE AH-3

FEED:	D.S. %	15.7
	pH	N/A
	ACID STRENGTH %	0.29
	ACID/WOOD %	1.59
	HCL (LBS)	1
	WATER (LBS)	339.4
	DRY SOLIDS (LBS)	62.8
	CELLULOSE (LBS)	37.99

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	226
RESIDENCE TIME (MIN)	1.44
% FLASH CALCULATED	21.53

PRODUCTS:

% D.S.	18.7
WATER (LBS)	284.94
DRY SOLIDS (LBS)	64.54
SOLUBLES (LBS)	29.95
INSOLUBLES (LBS)	35.59
CELLULOSE (LBS)	12.67
LIGNIN (LBS)	19.32
GLUCOSE (LBS)	20.39
LEVULINIC ACID (LBS)	0.99
5-HMF (LBS)	2.19
LACTIC ACID (LBS)	4.38

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	48.3
GLUCOSE/WOOD FEED %	32.47
CELLULOSE CONVERSION %	66.7

MASS BALANCE AH-4

FEED:		
	D.S. %	16.3
	pH	N/A
	ACID STRENGTH %	0.3
	ACID/WOOD %	1.54
	HCL (LBS)	0.88
	WATER (LBS)	294.91
	DRY SOLIDS (LBS)	57.05
	CELLULOSE (LBS)	35.31

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	220
RESIDENCE TIME (MIN)	2.25
% FLASH CALCULATED	20.44

PRODUCTS:

% D.S.	19.1
WATER (LBS)	252.07
DRY SOLIDS (LBS)	59.51
SOLUBLES (LBS)	31.06
INSOLUBLES (LBS)	28.45
CELLULOSE (LBS)	12.26
LIGNIN (LBS)	14.08
GLUCOSE (LBS)	18.55
LEVULINIC ACID (LBS)	0.89
5-HMF (LBS)	1.82
LACTIC ACID (LBS)	2

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	47.28
GLUCOSE/WOOD FEED %	32.52
CELLULOSE CONVERSION %	65.27

MASS BALANCE AH-5

FEED:		
	D.S. %	16.8
	pH	1.5
	ACID STRENGTH %	0.3
	ACID/WOOD %	1.49
	HCL (LBS)	0.85
	WATER (LBS)	284.78
	DRY SOLIDS (LBS)	57.12
	CELLULOSE (LBS)	38.61

ASSUME A CHEMICAL GAIN BASED ON 50% CONVERSION OF CELLULOSE

REACTOR CONDITIONS:

TEMPERATURE (DEG C)	220
RESIDENCE TIME (MIN)	1.89
% FLASH CALCULATED	20.37

PRODUCTS:

% D.S.	19.9
WATER (LBS)	239.74
DRY SOLIDS (LBS)	58.71
SOLUBLES (LBS)	23.76
INSOLUBLES (LBS)	35.8
CELLULOSE (LBS)	18.19
LIGNIN (LBS)	14.93
GLUCOSE (LBS)	15.06
LEVULINIC ACID (LBS)	0.59
5-HMF (LBS)	0.95
LACTIC ACID (LBS)	1.67
ACETIC ACID (LBS)	0.58

CONVERSIONS:

GLUCOSE/CELLULOSE FEED	35.11
GLUCOSE/WOOD FEED %	26.37
CELLULOSE CONVERSION %	52.9

APPENDIX V

FERMENTATION REPORT SUBMITTED BY

MORRIS WAYMAN

CONVERSION OF LIGNOCELLULOSICS TO ETHANOL

DSS File No. 51SZ.BDP
FINAL REPORT
by
MORRIS WAYMAN LIMITED
December 31, 1988
to
BIO-HOL DEVELOPMENTS

1 Introduction

This Final Report describes work performed during 1987 and 1988 under the above contract, or under different funding but contributing to the objectives of the contract. There were several parallel projects under way in the University of Toronto laboratory of Professor Wayman and it is difficult to sort out the origin and funding of each. In many cases, the first, crucial, truly pioneering experiments were made first, and paid for, outside of this contract. These include the first runs made using the Wenger cooker-extruder for prehydrolysis at the Sabetha, Kansas plant of Wenger, with aspen chips shipped down from Pembroke, Ontario in 1981, paid for out of an NSERC grant; the first prehydrolysis runs with SO2 catalyst in the Wenger cooker-extruder at Bio-Hol were paid for by Morris Wayman Limited; the first runs in the Stake reactor at the Universite de Sherbrooke with the system for adding gaseous SO2 to the steam inlet were made as a collaborative experiment between the two university groups; the first generation of R yeasts, which are the first efficient xylose-fermenting yeasts, were made under a grant to U of T by the Ontario Paper Company; the first runs with the continuous dynamic immobilized biocatalyst bioreactor precede this contract; and others. During the period under review, extensive work was done with enzymatic saccharification, simultaneous saccharification and fermentation, cellobiose-fermenting yeasts, and the use of _Zymomonas_ _mobilis_ for fermentation of wood hydrolysates, all in a grey area, which may or may not fall under the contract. Recent research with immobilized enzymes and a novel system for co-immobilizing enzymes and yeast is largely financed by the Ontario Ministry of Energy directly to U of T.

This Final Report makes no effort to sort out the financing of the various researches carried out at Professor Wayman's U of T laboratory, but will report all work done there which could reasonably be considered supportive of this contract's objectives. Monthly Progress Reports have been submitted which described the results and, in brief, the methodology, of both contract and non-contract research. Our laboratory has been open to the many visitors who came to inquire about our methods.

A particular source of curiosity by Bio-Hol has been our xylose-fermenting technique. We submitted "A Method of Generation of Efficient Xylose-Fermenting Yeasts" November 28, with the last page updated December 5, 1988. The Method Report states "Nothing about these methods is mysterious, or even sophisticated, or beyond the ordinary practice of any reasonably competent microbiologist." As with so many problems considered at one time to be very difficult, the answers are really quite simple. The Method Report contains all necessary information, and will be somewhat enlarged and clarified in this Final Report. A culture of the R yeast used in the Bio-Hol fermenter has been given to Bio-Hol for Weston, who, of course, have always been free to help themselves to the actively growing cultures in their fermenter at Bio-Hol.

We now understand clearly the biological mechanism of transformation of the appropriate culture collection yeasts into efficient xylose-fermenting R yeasts, the results of studies in 6 laboratories in 4 countries over 3 years, none of it paid out of this contract. A paper describing the scientific findings will be published in 1989, and immediately thereafter cultures of the R yeasts will be placed in appropriate public culture collections in the US, the UK and The Netherlands.

This Final Report is in two Parts. Part I discusses the progress made in specific areas of the work, such as prehydrolysis, etc. Part II contains experimental details, including primary analytical data. The following lists areas of progress for which we claim credit:

1. The use of the Wenger cooker-extruder for prehydrolysis.
2. The use of SO2 as prehydrolysis catalyst, a genuine breakthrough for coniferous woods.
3. The generation of R yeasts for efficient xylose fermentation, and tolerance of inhibitors.
4. Xylose fermentation techniques.
5. Inhibitor removal by steam stripping.
6. Fermentation of mixed prehydrolysate and hydrolysate.
7. The use of cellobiose-fermenting yeasts and mixed cultures for fermentation of incompletely hydrolysed biomass.

8. The use of Z. mobilis for fermentation of hydrolysates.

9. Enzymatic saccharification of SO2-prehydrolysed wood.

10. Simultaneous saccharification and fermentation of SO2-prehydrolysed wood; and high temperature fermentation.

11. Immobilization of cellulase enzymes and co-immobilization of enzymes and yeast for direct conversion of wood cellulose to ethanol.

12. The continuous dynamic immobilized biocatalyst bioreactor.

We have not mentioned here our genetic engineering work jointly with Allelix in which cellulase genes have been inserted into common yeast. Preliminary results were published in 1988, but the work is too early to be worth discussing here.

Some general comments and conclusions: some of these findings will find application far beyond the objectives of this contract. The methods used to generate R yeasts will affect many other areas of the fermentation industries. The continuous dynamic immobilized biocatalyst bioreactor will contribute to simplification and cost saving in those industries. The new immobilization technique developed under item 11 above is immediately applicable to starch fermentation, and will result in substantial saving in enzyme costs. Our very good results with Z. mobilis will support the efforts of the Lawfords and others who have trying for some years to interest alcohol producers in the use of that efficient fermenting organism.

The prospect for industrial production of ethanol from lignocellulosic biomass is now experiencing a bad press. This arises from two sources: the reported high cost of feedstock, and confusion over the best technology to use. Our survey of Ontario waste wood availability, and parallel surveys done in B.C. and federally show that there is adequate waste wood available at an acceptable price to supply a substantial fuel ethanol industry. A recent finding is that there is in Canada more than enough grain straw and corn stover surplus to agricultural needs to supply all of Canada's motor fuel as ethanol, all simply for the cost of gathering, estimated conservatively at $15.00 per tonne, or 4 c/L. That should settle the question of feedstock.

To address the confusion over technology, we have prepared as the last section of Part I an identification of the ideal biomass process from a comparison of the many now proposed. We conclude from this comparison that the ideal process is quite simple: straw or stover or sawdust is prehydrolysed with SO2 in appropriate equipment - at least five effective designs are available - and then without separation of hemicellulose are simultaneously saccharified by enzymes and fermented by R yeasts, or if saccharification is incomplete, by a mixed culture of R yeasts and a cellobiose fermenter, of which several are available. Since R yeast functions well at over 40 C, SSF presents no problems. Finally, of course, conventional distillation and dehydration. Our principal recommendation would be that further development of this process should be carried out with straw as feedstock. This differs from the Iogen or IFP processes in that we make xylose ethanol, which improves the economics enormously.

Harold Innis, who was Marshall McLuhan's intellectual forefather, once quoted an old Greek who said "Minerva's owl flies only in the dusk," which translated into modern idiom reads "Too late we get too smart." It is not too late. As long as the US imports 40% of its oil requirements, and that percentage is increasing, there is an important and valued place for the fuel ethanol industry.

2. Prehydrolysis equipment and conditions

The conditions for prehydrolysis are reasonably well defined, and at least five alternative kinds of equipment are available industrially. These are the SLR reactor, the Stake reactor, the Wenger cooker-extruder, the Masonite gun and the Sunds Defibrator. All five are based on short time treatment with steam or dilute acid at elevated temperature and pressure. The first three have been studied here.

The SLR reactor is very good for finely divided feedstocks. Thus for cobs, straw or other agricultural residues which are relatively easy to grind the SLR is perhaps better than any of the others for prehydrolysis. Temperatures of 160 - 200 C are reached in less than 1 minute, and holding times are 3 - 10 minutes. The catalyst used is HCl. It will not accept conventional wood chips, and wood-grinding equipment to reduce wood chips to pass 20 mesh or better is far too energy consuming and expensive.

Our experience in prehydrolysing wood chips with the Stake reactor goes back to 1977. The Stake reactor has been used to prehydrolyse both hardwoods and softwoods in research carried out in collaboration with Professor Chornet's group at U. de Sherbrooke, where there is a Stake reactor. A system for adding gaseous SO2 with the inlet steam was installed and used as catalyst for prehydrolysis. The products were taken to the U of T laboratory where they were enzymatically saccharified and the sugars fermented to ethanol in very good yields. The Stake reactor is a large and expensive piece of equipment. It works well, but the product is not uniform. The internal helix acts as a conveyor but not as a mixer, nor does it provide attrition. The discharge port permits rather large pieces of wood to pass. As a result the product requires defibering to reduce particle size for further processing. We are told that the group at the IFP pilot plant at Soustons, France, has had the same experience. However, the energy requirement for defibering the pretreated wood is low. For prehydrolysis, a combination of the Stake reactor, perhaps with an improved, broken flight helix, and an attrition device, is a strong candidate.

The Wenger cooker-extruder has a built-in attrition action. It accepts standard wood chips - it may prefer somewhat smaller chips - and sawdust. It reaches 200 - 210 C in a few seconds, and has a retention time of 45 - 90 seconds. This is just fine for prehydrolysis. The feed should be at 35 - 50+% moisture for smoothest operation. The product is lacey, fibrous and very well suited to further processing, either by the SLR or enzymatic. The structure is so fine that we have used it for SSF with co-immobilized enzyme and yeast.

The operating conditions for the Stake and the Wenger can vary over some wide range of temperature, pressure and amount of catalyst, but in both reactors care must be taken not to overcook. This probably applies equally to all five reactors being discussed. When wood is overcooked in prehydrolysis, sugars so set free react in two different directions: they dehydrate to furfurals, and they condense with lignin. Both reactions reduce yields of hemicellulose sugars, and inhibit further hydrolysis reactions. There is in each case an upper limit for time-temperature which must not be exceeded. Overcooked wood is generally dark in colour and adherent into lumps. With the Wenger, the condition can be recognized and corrected quickly.

The usual product of the Masonite gun installation at Iogen is autohydrolysed aspen made with steam at 230 - 235 C for 45 - 90 seconds. While the Masonite gun is often referred to as a batch process, this is an unfair designation, since banks of such pressure vessels are automated so as to accept a nearly continuous feed and provide a nearly continuous discharge. A better designation would be a rapid cycle reactor. The heavy high pressure vessels are made to operate at up to 1200 psig and are 30 cu ft in volume. The product needs attrition and then is quite suitable for enzymatic saccharification. While it may never have been used with coniferous woods, there is no apparent reason why it cannot be a useful prehydrolyser for all feedstocks.

The Sunds Defibrator accepts chips and by a combination of mechanical action and steam, separates the fibres to the extent necessary for making fibreboard. The Defibrator installed at TVA is lined with a zirconium alloy to resist corrosion, which doubled the already considerable cost. This is because TVA insists on using sulphuric acid catalyst, which is highly corrosive. The standard equipment is used world-wide in hundreds of installations and could undoubtedly be used for prehydrolysis with SO2 catalyst. It may have a lower power consumption than either the Stake or the Wenger.

All five designs of prehydrolysis equipment are serious candidates for the development of biomass conversion, the choice among them to be made on an engineering basis. No one of the five stands out as being far ahead of the others in performance.

3. The use of SO2 as prehydrolysis catalyst

Any inorganic acid can be used as prehydrolysis catalyst. Sulphuric acid is far too corrosive, while HCl has been shown by SLR to be excellent in their particular alloy. No Wenger, Stake, Masonite or Sunds equipment has been fabricated with SLR's alloy. However, the use of SO2 in prehydrolysis essentially avoids corrosion when using a conventional good grade of stainless steel. There are many decades of experience in using SO2 for grain steeping and wood pulping so the technology of its production, packaging and application are highly developed. As a weak acid it provides adequate H+ for prehydrolysis without over acidifying. As a gas, it distributes itself well through the moist wood. It is cheap, and has proven itself to be a good choice.

The use of SO2 for prehydrolysis was first reported from this laboratory in 1983-84. There is an earlier history which goes back to 1909, when George Tomlinson I built two ethanol from wood plants in the southern US using SO2 for hydrolysis. The first application in a continuous process was carried out in the Stake reactor at the U de Sherbrooke, published in 1986 with Estaban Chornet and Ralph Overend co-authors with the U of T investigators. Prof. Chornet's group devised the system for adding gaseous SO2 to the Stake reactor.

We are somewhat surprised at the slow adoption of this simple, effective method by investigators in other countries, but expect that IFP and others will be using SO2 in prehydrolysis as soon as they start to use coniferous sawmill wastes. While the use of SO2 is a genuine breakthrough for coniferous woods, the advantages for all feedstocks are so great that once tried they will never go back.

4. The generation of R yeasts

The importance of xylose fermentation when making alcohol from wood or from agricultural residues is great, and can make the difference between success and failure. We have estimated the contribution of xylose as a percentage of ethanol produced to be, for pine 28%, aspen 43%, corn cobs 66%, wheat straw 65% and bagasse 53%.

Our success in generating yeast strains which are effective and efficient xylose fermenters began with Dr. Shiyuan Yu's Ph.D. research, which was funded by the Ontario Paper Company. His mission was to eliminate residual sugar from the stillage at OPC's spent sulphite liquor (s.s.l.) alcohol plant at Thorold. Stillage from the plant was used to make vanillin. By removing residual sugar, Dr. Yu hoped to simplify and improve the vanillin manufacturing process.

There are many yeasts which ferment xylose, but none of these when in the wild state, that is as received from culture collections such as the American Type Culture Collection (ATCC), or Centraalbureau voor Schimmelcultures (CBS), are rapid or efficient xylose fermenters. They would not ferment solutions with more than 2% xylose, and were very slow at that. In 1984 we were given a culture of _Candida shehatae_ ATCC 22984 by Dr. Thomas Jeffries of the USDA Forest Products Laboratory, Madison, Wisconsin, which, although still a weak xylose fermenter, was better than any strain to that date. Dr. Yu found that this _C. shehatae_ (_C.s._) would ferment the xylose in s.s.l., but that fermentation was slow and incomplete in a reasonable time. However, he found that if he used as inocula not fresh cultures but yeast from a preceding culture, the results were somewhat better. He therefore began recycling the yeast repeatedly, up to a total of 72 times. At that time, the adapted or acclimatized yeast, now called C. shehatae R, proved to be an efficient xylose fermenter.

When Dr. Sarad Parekh and Dr. Rannade Parekh joined us late in Dr. Yu's studies, they undertook a survey of other yeast species and strains said to be xylose fermenters, especially those suggested by investigators in South Africa and The Netherlands. Among the many studied, they identified Pichia stipitis CBS 5776 (P.s.) to be the most efficient. In their hands, 10 - 12 recycles were sufficient to obtain the desired effect. In a comparison of C.s. and P.s. and the corresponding R strains, P.s. has shown itself to be marginally better than C.s. As a result, subsequent studies have used P.s.R almost exclusively.

There may be reservations about the conclusions to date. P.s. may not be the ultimate xylose fermenter, and others will be found to be even better. Also the adoption of 10 - 12 recycles may not be the optimum. While there is hesitation about reverting to the tedium of 72 recycles, the resulting R culture is stable and can be preserved and reused, so the effort to go to more recycles - to 20? 40? 72? - may be worth it, especially when badly inhibited substrates are being fermented. Also, a continuous acclimatization method proposed by Dr. Walter Steiner, of Innsbruck, can probably achieve the same result while keeping a steady supply of fresh R yeast available at all times, but this has yet to be proven.

To illustrate the difference, Steven Tsuyuki reported from this laboratory that Jeffries' yeast would ferment only 50% of a 140 g/L xylose solution in 48 h, confirmed by Sarad Parekh, who showed that after adaptation this was completely fermented in the same time.

We have not until recently understood the mechanism of this adaptation but we are now very clear about the physiology, the enzyme profiles, the morphology and the genetics. It is now established that the R yeasts are polyploid, while the culture collection yeasts are haploid, with P.s. having a slightly higher ploidy than C.s. This discovery has far-reaching consequences for the fermentation industries. It has long been known that industrial "robust" yeast strains are polyploid, but this is the first demonstration of the reverse: that artificial induction of polyploidy results in robust, improved performance.

5. Xylose fermentation technique

In what follows, methods of inducing efficient xylose fermentation are described. It should be emphasized that nothing about these methods is mysterious or even sophisticated, or beyond the ordinary practice of any reasonably competent microbiologist.

In addition, there are here useful descriptions of conditions peculiar to xylose fermentation, and some examples of fermentation of xylose and wood hydrolysate solutions.

5.1 Induction of R yeasts

Obtain fresh cultures of _Pichia stipitis_ CBS 5776 and _Candida shehatae_ ATCC 22984 from their respective culture collections and transfer to liquid medium. Check for contamination: we have occasionally found bacterial contamination in such cultures, which then need to be discarded and fresh cultures ordered.

With fresh cultures, and with stock or maintenance cultures, it is good practice to prepare streaked plates and subculture from single cell colonies to ensure specific purity. Also stock cultures should be transferred routinely every 2 months.

The liquid medium has xylose only as carbon source (40 g/L) and YMP nutrients (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone), pH 5.4, grown in 50 mL medium in 125 mL conical flasks, or in 100 mL medium in 250 mL conical flasks with porous plug stoppers. When good growth is obtained - with fresh cultures there may be a long lag period, whereas with cultures maintained as follows, 24 h is sufficient - transfer a loopful to YMP agar slants with xylose as the carbon source to form stock or maintenance cultures, and sources of future inocula. Adapted cultures are maintained in the same manner.

Wood prehydrolysates, especially aspen, are the medium of choice for adaptation, although mixed prehydrolysate hydrolysate or spent sulphite liquor or other stressful substrates can be used. Aspen prehydrolysates prepared in the Wenger using 1.5 - 3.0% SO2 on dry fibre are rich in xylose, about 70% xylose to 30% glucose, and contain in addition wood derived inhibitors including furfurals from pentose sugars, phenolic lignin fragments, and acetic acid. The acetic acid concentration is the aspen prehydrolysates is too high for successful growth and needs to be reduced either by dilution or by steam stripping to 0.5g/L (NOT percent) or less. Total sugars for this purpose should be about 40 g/L. The pH should be raised with lime to 5.5, and as nutrients, 0.2 g/L urea and 0.1 g/L diammonium phosphate (U-DAP) are added.

It might be thought that the yeast should be adapted to the specific substrate for which it is to be used, for example pine prehydrolysate instead of aspen. This option is open, but it is unlikely to be as effective as the use of the more toxic aspen prehydrolysate (or s.s.l. or other severely inhibited solution).

For adaptation, the yeasts are cultivated in 100 mL steam stripped aspen prehydrolysate fermentation medium, pH 5.5, in 250 mL conical flasks on a rotary shaker at 150 rpm at 30 C for 25 h, using porous plug closures. Cells are harvested by slow (1000 rpm) centrifugation, twice washed by suspension in saline and recentrifuging, and the resulting pellet is inoculated in fresh prehydrolysate fermentation medium. Cell concentration should be in the range of 8 - 16 g/L. This procedure, that is recovery and recycling of the yeast, is repeated at least 10 times, preferably more.

The adapted yeast is inoculated into YMP agar slants with 2% xylose for stock cultures. It should be checked for xylose fermentation rate and effectiveness, and be plated out as a check against purity.

Alternatively, there is a continuous acclimatization method which requires a plentiful supply of fermentation medium, that is steam stripped aspen prehydrolysate with U-DAP or YMP. A 1 L continuous fermenter is set up with a controlled slow flow of medium and a high inoculum, 12 - 15 g/L. A slow flow of air is provided. Flow of medium is set at such a rate that the cell concentration in the fermenter is constant. After 1 to 4 weeks of such operation, the culture should be well adapted to the medium. If the laboratory or plant is running fermentation constantly, or frequently, there is no reason to discontinue or interrupt the adaptation, which does not require much attention. However, if contamination is observed, it should be stopped and cleaned up. It should be stressed that this method has given rather mixed results, some very good, some not so good. It requires further development.

5.2 Fermentation conditions

5.2.1 Nutrients

For inoculum preparation and for culture maintenance, we use YMP medium and for fermentation U-DAP medium. Recently we have substituted corn steep liquor for U-DAP with complete success. This needs further study, but we can see many advantages in using sterile corn steep liquor as nutrient. As the work develops we may make this changeover complete.

The level of nitrogen nutrient influences ethanol yields. Higher levels favour cell synthesis and lower alcohol yields, while lower levels restrict alcohol production. Also the requirement of N depends on the concentration of sugars, and the cell concentration, in the fermenter.

5.2.2 pH

The pH may be as low as 4.0 or as high as 6.5. We prefer 5.5. If the starting pH is too low, it may fall below useful levels during fermentation.

5.2.3 Anti-foam

We use 10 - 20 ppm polypropylene glycol when required.

5.2.4 Cell concentration

Cell concentration should be high, as found by Tsuyuki, by Yu and by Parekh. We prefer 8 - 16 g/L freeze-dried weight for fermentation. Still higher cell concentration would be still better, giving faster fermentation and less diauxie. This reaches its maximum with immobilized cell fermentation as repeatedly demonstrated by very fast fermentation in the continuous dynamic immobilized biocatalyst bioreactor.

5.2.5 Air flow

Both *C.s.* and *P.s.* require the presence of some air - "semiaerobic" fermentation - for efficient xylose fermentation. The amount required varies with the composition of the substrate, particularly the xylose and glucose concentrations, and varies during the course of a batch fermentation. In a medium high in glucose, such as combined prehydrolysate-hydrolysate, air flow should be low to start, until all glucose is consumed, anad then increased, and tapered off towards the end of fermentation when xylose concentration is low. In 70:30 glucose:xylose an air flow configuration might be 0.15 vvm (volumes of air per volume of medium per min) for 12 - 16 h, the 0.25 - 0.30 vvm for 16 - 24 h, the 0.07 - 0.10 vvm until fermentation is complete. When the sugar ratio is reversed, that is 30:70 glucose:xylose, air flow may be 0.20 - 0.30 vvm through most of the fermentation, but reducing it near the end. In conical flasks of any size, 40 - 60% of the space should be occupied by the medium, porous plugs should be used as closures and rotation should not be too vigorous.

5.2.6 Inhibitors

Acetic acid is the most serious inhibitor in processing aspen and agricultural residues. Acetic acid in concentrations above 0.5 g/L (NOT %) slows fermentation rate seriously. Below that concentration it may act as a modest carbon source. We have been able to ferment higher acetic acid concentrations, but the lag periods were about 8 days. It may be possible to adapt these yeasts to tolerate high acetic acid concentrations, or even to ferment them, thereby avoiding steam stripping, but this has not been explored. Acetic acid is volatile in steam at atmospheric pressure or above, but NOT at low pressure, and its removal by steam stripping is rapid and effective. At the same time other volatile inhibitors such as other volatile fatty acids and the furfurals are reduced in concentration. Until a better method is established, steam stripping is necessary.

This is not always completely successful. We have found prehydrolysates and hydrolysates containing powerful inhibitors. Dilution frequently cures the problem, for example when aspen prehydrolysis is followed without washing by enzyme saccharification, no steam stripping is necessary, the dilution due to the enzyme treatment being sufficient to overcome the inhibition. Alternatively, these inhibitors can sometimes be removed by active carbon treatment.

Since feedstock composition and prehydrolysis and hydrolysis conditions vary, the compositions of the wood sugar solutions vary, including the quantity and type of inhibitors. Successful fermentation of some of these solutions will be strongly influenced by the levels of these inhibitors. This may limit the concentration of the hydrolysate which can be fermented. In exploratory runs, an upper limit of 120 g/L total wood sugars should be adopted, and successful fermentation should yield 6% ethanol by volume in an acceptable time. If not successful, serial dilutions should be undertaken to establish at what concentration the solution is not inhibited.

If serial dilution does not succeed in establishing a useful fermentation, the next step would be to adapt the yeast(s) to this particular set of inhibitors.

We have found in some cases that a refractory hemicellulose solution may be fermented efficiently if the glucose level is raised from about 25 - 30% of total reducing sugars (TRS) to 50%.

A high ash level, such as results from NaOH neutralization of acid-catalysed hydrolysis, slows fermentation rate and lowers the yield of ethanol. In the presence of high salt levels, the yeasts, in order to protect themselves against osmotic effects, synthesize glycerol and xylitol. This diverts part of the sugar to polyol production and away from ethanol production. Deashing by means of ion exchange would decrease this problem, or perhaps the use of ammonia for neutralization, as is now practiced in sulphite alcohol plants.

There is an upper limit to glucose tolerance by these yeasts. In one set of runs where initial TRS was 250 g/L and glucose:xylose was 70:30, fermentation slowed markedly when ethanol concentration exceeded about 9% by volume. Removal of the ethanol by vacuum distillation at low temperature enabled fermentation to continue satisfactorily to completion.

5.3. Yield calculations

Theoretically, 1 kg glucose should yield 515 g ethanol (650 mL), and 1 kg xylose should yield about 470 g ethanol (595 mL). Because of formation of cells, glycerol and other co-products, theoretical efficiency is never obtained, and 90% efficiency has to be considered quite satisfactory.

5.4. Many examples of xylose fermentation are given in Part II of this Final Report. A summary of our findings is given here.

5.4.1 Xylose

in shake flasks, Progress Report No. 5 (June 1987) and in Appl. Microbiology and Biotechnology 25, 300 - 304 (1987), 105 g/L xylose, *P.s.* R and also *C.s.* R, 88 -95% of the xylose fermented in 96 h, 0.42 g ethanol/g xylose consumed;

in 4 L fermenter, PR 8 (Sept 87), PR 13 (Feb 88), 28 g/L xylose, *P.s.* R, 95% of xylose consumed in 32 h, 0.49 g ethanol/g xylose consumed;

in 28 L fermenter, PR 14 (Mar 88), PR 15 (Apr 88), PR 17 (Jun 88), 34 - 56 g/L xylose, P.s. R, 88 - 94% of xylose fermented in 48 - 64 h (mostly spent in building up cell concentration), 0.44 - 0.47 g ethanol/g xylose consumed.

5.4.2 Xylose-Glucose solutions

in shake flasks, PR 8 (Sept 87) P.s. R, 70:30 glucose:xylose, complete fermentation in 24 h, 0.48 g ethanol/g sugar consumed.

in 4 L fermenter, PR 13 (Feb 88), P.s. R, 45 - 62 g/L TRS, 70:30 glucose:xylose, 85 - 99% consumed in 34 - 45 h, 0.43 g ethanol/g sugar consumed.

in 28 L fermenter, PR 18 (Jul 88), P.s. R, 130 g/L TRS of which 38 g/L xylose, cell concentration buildup for 48 h, then fermentation for 44 h to give 6.9% ethanol by volume, 95% of sugars fermented, 0.44 g ethanol/g sugar fermented.

5.4.3 Prehydrolysates

in shake flasks, PR 8 (Sep 87), PR 9 (Oct 87), PR 12 (Jan 88), PR 16 (May 88), steam stripped Wenger SO2 prehydrolysates of aspen, 23 - 37 g/L TRS, and of pine, 42 g/L TRS, glucose:xylose about 70:30, P.s, R very good xylose utilization in 36 h, 0.47 g ethanol/g sugar consumed.

in 4 L fermenter, PR 13 (Feb 88), PR 17 (Jun 88), PR 18 (Jul 88), P.s. R, steam stripped, 56 g/L TRS, 83% xylose and 100 % glucose fermented in 41 h, 0.47 g ethanol/g sugar fermented.

in 28 L fermenter, PR 21 supplement (Dec 88), aspen, steam stripped, diluted to 60 g/L including 40 g/L xylose, a xylose concentration equal to the xylose content of a mixture of prehydrolysate and hydrolysate with 130 g/L TRS, P.s. R, 85% of xylose and 100% of glucose fermented in 68 h, 0.44 g ethanol/g sugar fermented.

5.4.4 Mixed Prehydrolysate-Hydrolysate

in shake flasks, PR 12 (Jan 88), aspen and pine Wenger SO2 prehydroysed followed by enzyme saccharification without washing or separation of prehydrolysate and hydrolysate, and without steam stripping, P.s. R, 38 - 53 g/L TRS, 36 h, 80 - 100% utilization of xylose, 0.46 -0.48 g ethanol/g sugar consumed.

in 4 L fermenter, PR 18 (Jul 88), PR 19 (Aug 88), Wenger SO2 prehydrolysed pine hemicellulose sugars separated and steam stripped, then added to hydrolysate made by HCl-catalysis in the SLR reactor, P.s. R, 102 - 105 g/L TRS including 25.0 g/L xylose, 36 - 41 h, 91 - 99% of sugar consumed, 0.42 - 0.45 g ethanol/g sugar consumed.

in 28 L fermenter, PR 21 (Nov 88), aspen mixed SLR hydrolysate and steam stripped Wenger prehydrolysate combined, 116 g/L TRS including 35 g/L xylose, acetic acid less than 0.5 g/L, fermented by 10 g/L P.s. R, aeration 0.15 VVM for 24 h, then 0.24 VVM for 24 h, then dropped to 0.12 VVM. Of the total sugars, 93% were consumed, including 86% of the xylose, ethanol produced was 6.0% by volume, 0.43 g ethanol/g sugar consumed.

6. Removal of inhibitors by steam stripping

Prehydrolysis of biomass results in formation of inhibitors. If prehydrolysis conditions are properly chosen and the wood, etc., is not overcooked, these can be dealt with by steam stripping or other mild methods. If the biomass is overheated much above 200 - 210 C, even briefly, the inhibitors formed may be impossible to deal with.

Inhibitors are of three kinds: furfurals formed by dehydration of sugars, phenolic lignin fragments and acetic acid. Of these, acetic acid is best understood. At concentrations of 0.2 g/L or less, acetic acid is assimilated by the yeast, thus sparing sugar for ethanol formation. At 0.6 g/L or more, fermentation slows down, and at 1 g/L or more, may be so slow as to be impractical. Between 0.3 g/L and 0.5 g/L, the rate is tolerable, but not too healthy.

Acetic acid is steam-volatile and is removed rapidly by steam stripping at atmospheric pressure or above. However, the boiling point of acetic acid is 118 C, and it is not steam volatile at low temperatures. During vacuum evaporation, at temperatures in the neighbourhood of 50 C, the acetic acid in solution becomes more concentrated. If the prehydrolysate needs concentrating, this should be done at atmospheric pressure by boiling off excess water, or, if that results in unacceptable sugar destruction, by a two-stage treatment, vacuum evaporation followed by steam stripping.

There are other means of removing acetic acid such as activated carbon, ion exchange resins, and even simpler methods. We have found that on prolonged fermentation, yeast becomes somewhat adapted to acetic acid in a week or two. This and other chemical methods will be explored in the next phase of this work.

7. Fermentation of mixed prehydrolysate and hydrolysate

All proposed processes for fermentation of wood sugars prior to the beginning of this work at the U of T laboratory have called for two separate fermentations, of prehydrolysate with marginal yeasts such as Pachysolen tannophilus and hydrolysate with common yeast. This is no longer necessary. The R yeasts are efficient fermenters of xylose, glucose and mannose, the principal wood sugars. It is not necessary to provide two fermentation systems, expensive to install and, with different yeasts, to operate. Prehydrolysates and hydrolysates are now fermented in one vessel with one yeast. This represents a substantial advance, and enables a considerable saving from process simplification.

When acid hydrolysis of wood cellulose follows prehydrolysis, it is necessary to separate hemicellulose sugars from the residual cellulose, since they would otherwise be destroyed in the more drastic conditions of acid cellulose hydrolysis. Then there are two solutions available which may be treated separately if that is advantageous, for example if crystalline xylose or furfural are to be products, or they may be fermented together. When enzyme saccharification of the cellulose of prehydrolysed wood is used, it is not necessary to wash the hemi sugars from the residual cellulose, the whole mass being treated together by enzymes in one step. This step may be part of combined, simultaneous saccharification and fermentation (SSF). Then the total process becomes very simple: prehydrolysis followed without washing by SSF, then of course distillation and dehydration.

8. Cellobiose fermentation and mixed cultures

In the course of this research we have identified several yeasts which are efficient cellobiose fermenters, particularly Candida lusitaniae, Brettanomyces clausenii and Torulopsis wickerhamii. The importance of these yeasts lies in that cellobiose, a normal product of cellulose saccharification, is a repressor of cellulase activity, preventing complete saccharification, and accumulating cellobiose in place of glucose. When cellobiose is removed by fermentation by one of these yeasts, saccharification proceeds more rapidly and more completely.

These yeasts are not good xylose fermenters, and for efficient fermentation of wood sugars a mixed culture of one of the cellobiose fermenters and a xylose fermenter has been successful.

Until now all enzyme saccharification has required the addition of the enzyme beta glucosidase along with the cellulose to hydrolyse the cellobiose. This is no longer necessary.

Furthermore, saccharification need not be complete, and some savings are realized from incomplete saccharification. At least T. wickerhamii and to a lesser extent the other two are able to ferment low DP cellodextrins.

9. Zymomonas mobilis

We have about-faced from skepticism to enthusiasm: Zymomonas mobilis is an efficient, rapid fermenter of wood cellulose hydrolysates. In the event that hydrolysates are, for good reasons, to be fermented separately from prehydrolysates, we consider Z. mobilis to be a good candidate. We have had excellent, rapid fermentation and high yields with free cells and with immobilized cells of this glucose and mannose fermenting bacterium.

10. Enzymatic saccharification of prehydrolysed wood

We have consistently had higher yields of ethanol when wood cellulose, or agricultural residue cellulose, has been saccharified with enzymes than on acid hydrolysis. This difference arises in part because acid hydrolysis of cellulose cannot be considered a mature technology. In publications from 1978 we reported laboratory, small scale experiments which gave high ethanol yields when acid hydrolysis was carried out in 4 successive stages, each being a minimal partial hydrolysis. Equipment to separate sugars from cellulosic residue as formed would accomplish the same high yields, but no such equipment is available.

In enzymatic saccharification of prehydrolysed wood, with or without prehydrolysate present, a mixture of enzymes is normally used, based on cellulase, or when prehydrolysis is incomplete, including hemicellulase, and until recently, beta glucosidase. In addition, we add promoters in the form of trace elements, Vitamin B12 and a surface active agent such as Triton X100, as described in our 1986 publication with Professor Chornet's group. The enzymes are added to a slurry of cellulosic residue and mildly agitated at 45 - 50 C. Saccharification is complete in 48 hours.

There is now no significant resistance in R & D planning to enzyme saccharification. Reluctance to consider this process arose from the purported high cost of enzymes, while now at least 10 companies in 6 countries make and sell cellulase. Our supplies used to come from Denmark, and now are mainly from Japan and Finland. Furthermore, IFP, Iogen and others have found it remarkably easy to make their own enzymes in-plant, a practice not unknown in the grain alcohol industry. A recent study by John Wright, of SERI, concluded that enzyme production costs are 2 - 3 c/L ethanol when used in SSF. Enzyme costs become even less when immobilized enzymes are used (see 11 below).

The availability of cheap cellulase changes the concept of bioconversion of wood to ethanol quite dramatically, and needs to be given due weight in research and development planning.

11. Simultaneous saccharification and fermentation of prehydrolysed wood, and high temperature fermentation

By adding enzymes and yeast together - or after a short period of enzymes only first, as is common in starch SSF - the total time required for conversion of wood cellulose to ethanol is much reduced. The principal difficulty has been the disparity in optimal temperatures between enzyme activity and fermentation, the former being 45 - 50 C, and the latter 30-32 C. We have found satisfactory performance at 37 C with R yeast. We are now informed by CBS that this yeast ferments well at 41 C. This would eliminate any concern over the temperature gap, and our future SSF runs will be made at this higher temperature. (Investigators have been searching avidly for higher temperature yeasts for a very long time, and here we have one in hand!)

We are also informed, by SERI, that SSF much reduces the amount of enzyme required for saccharification. This would be an added advantage.

If lignin recovery becomes economically attractive, the overall process has to change to include removal of lignin after prehydrolysis and before SSF.

12. Immobilized cellulase enzymes and co-immobilization of enzymes and yeast for direct conversion of wood cellulose to ethanol

Preliminary work carried out under an Ontario Ministry of Energy grant to U of T has shown that delignified aspen wood which has been prehydrolysed in the Wenger is readily saccharified by immobilized enzymes to sugars, and these have been fermented to ethanol. The wood so prepared can also be SSF'd to ethanol by co-immobilized cellulase enzyme and common yeast. A new method of co-immobilization has been shown to be quite effective. This work will be reported at the Bioenergy R & D Seminar in Ottawa in April. These immobilization procedures result in significant savings in enzyme requirements.

In this method, there is a reaction between two solids, the aspen cellulose and the immobilized enzyme. We attribute its success in large measure to the fine physical form of the Wenger product.

13. The continuous dynamic immobilized biocatalyst bioreactor

This wonderful fermenter was described and exhibited in action at the Bioenergy R & D Seminar in Vancouver in February 1987. We believe it represents a significant step forward in fermentation technology. With it, we have obtained very rapid 20 minute complete fermentations, a sight to see. We have also used it with immobilized yeasts, Z. mobilis, cellulase and cellulase + yeast. It would be well worth the effort for someone in the fermentation industries to build a pilot model.

14. An ideal process for conversion of cellulosics to ethanol

An ideal process for conversion of cellulosics to ethanol would have to have inexpensive, compact feedstock to begin with. The processing then should involve as few items of major equipment, that is as few stages, as possible, produce good yields of fermentable sugars at high concentrations, produce few inhibitors so the fermentation may proceed rapidly and completely, to give ethanol at 6% or more by volume in solution for economical distillation. It should also have as little waste products as possible to be disposed of in the environment.

Processes for conversion of cellulosics to ethanol have many unit operations to choose from: autohydrolysis versus prehydrolysis; separate recovery of xylose and lignins or fermentation of xylose and the hexoses together leaving lignin recovery to follow saccharification; acid hydrolysis versus enzyme hydrolysis; separate enzyme saccharification versus SSF, whether to leave the distilled alcohol at the 95% mark, or to dehydrate it to 99+% alcohol. The choice among these alternatives depends on the intended products of the plant and its feedstock. For example, deciduous woods may be processed by either autohydrolysis or by prehydrolysis, while coniferous woods are unresponsive to autohydrolysis. Xylose and lignin may be separated and upgraded when there is a market for their products, otherwise xylose may be left in place during enzyme saccharification, although not during acid hydrolysis, and in either case fermented together with the glucose of cellulose hydrolysis. If the glucose is to be fermented separately, there may well be an advantage in using Z. mobilis rather than S. cerevisiae. Choices such as to make or buy enzymes, and whether to dehydrate the ethanol are easier, the former depending upon economics, including investment, and the latter upon the specific markets for the ethanol.

In the following Table 1 these options are listed and combined in various ways, and from these combinations the processes most useful for specific feedstocks and products are selected. From these the simplest, ideal process for ethanol from cellulosics is indicated. The possibility of using genetically engineered yeasts for SSF is not listed because such yeasts effectively do not exist as yet, although some optimists, including the authors, believe they will come into use in this century.

Process No.1 is exemplified by the Scholler process; Process No.2 requires the least investment per litre of alcohol produced; Process No.3 is exemplified by the St. Lawrence Reactors/Biol-Hol process; Process No.4 by the Madison-TVA process; Process No.5 is designed for the greatest value of by-products; and Process No.6 is exemplified by Iogen or by Soustons.

We would nominate process No.2 as the simplest and best: prehydrolysis followed without any separation of hemicellulose or lignin directly by SSF, and then of course, distillation, which is common to all processes, and dehydration if the product is to be blended with gasoline or used for industrial alcohol. This process gives the highest yield of ethanol for the least investment, and it can be designed to be continuous. Indeed, the parallel to grain processing is quite marked, as shown in Figure 16.14. In conversion of corn to ethanol, the engineer has the option of dry grinding with minimum recovery of co-products such as gluten, or wet grinding with maximum recovery of co-products. In processing cellulosics the same option exists: Process No.5 allows for the recovery of xylose and lignin as well as ethanol. As shown, it provides for autohydrolysis rather than prehydrolysis because of the greater yield of lignin, but then it cannot be used for coniferous woods.

The acid hydrolysis processes, Nos. 1, 3 and 4, are given a lower rating because of low yields of ethanol, and the need to extract hemicellulose sugars between prehydrolysis and hydrolysis. This may, of course be an advantage if there is a market for xylose; and while not shown, lignin may also be extracted between prehydrolysis and hydrolysis. The Iogen and Soustons processes are designed for deciduous woods and agricultural wastes. In both cases, there is no evident reason why SO2 catalyst cannot be used and the biomass resource base thereby broadened. Also their separate enzyme saccharification and fermentation can, if desired, be combined. In a word, these processes can readily be modified to resemble more closely the ideal process, Process No.2. The expectation would be that with a suitable biomass supply at an acceptable cost, both the technology and the economics of our chosen process will be shown to be quite competitive with petrol for motor fuel, and that a substantial new industry will be based upon it.

Table 1 Process configurations for conversion of cellulosics to ethanol

Process No.-> Unit Operation	1	2	3	4	5	6
1. Prehydrolysis	-	+	+	+	-	-
2. Autohydrolysis	-	-	-	-	+	+
3. Hemicellulose extraction	-	-	+	+	+	+
4. Lignin extraction	-	-	-	-	+	+
5. Acid hydrolysis	+	-	+	+	-	-
6. Enzyme saccharification	-	-	-	-	-	+
7. SSF	-	+	-	-	+	-
8. Enzyme production	-	+	-	-	+	+
9. Ferment xylose,glucose separately	-	-	±	±	-	-
10. Ferment xylose, glucose together	+	-	±	-	-	+
11. Distillation	+	+	+	+	+	+
12. Dehydration	±	±	±	±	±	±
Total plus	3	4	5	5	6	7

15. Fermentation process optimization

Fermentation conditions, that is microorganism preparation, nutrients, pH, antifoam, cell concentrations, air flow and inhibitors have been discussed in Section 5. It cannot been said that any of these conditions has been truly "optimized" since absolute optimization is impossible when substrates such as wood prehydrolysates and hydrolysates are so variable. Their compositon depends upon wood processing conditions and wood species. In this section we shall expand our previous comments on aeration, pH and temperature, and leave to Section 17 discussion of conditions for industrial fermentation to be expressed in process design.

15.1 Aeration.

In fermenting glucose solutions or wood hydrolysates free of xylose, no aeration is required, fermentation to ethanol being anaerobic. When solutions containing xylose are to be fermented, "semiaerobic" conditions are required. These are readily defined in shake flasks: 50 ml of medium in 250 ml conical shake flasks with a foam closure provide the necessary amount of air for semiaerobic fermentation. Under these conditions, xylose is readily fermented by R yeasts, especially in the presence of glucose. When both xylose and glucose are present in shake flasks, no diauxic effect is noticed, that is, glucose and xylose are fermented simultaneously. However, in 4 L and 28 L fermentations, glucose is fermented almost completely before xylose fermentation becomes significant. Sometimes this is not a true diauxic effect, xylose fermentation in the presence of inhibitors being significantly slower than glucose fermentation.

The best aeration rate depends upon the composition of the substrate and it varies during the course of any given fermentation. The principles of choosing aeration rates are as follows. In the first phase of fermentation, when only glucose is being fermented, aeration is not needed, or the rate should be low. When the glucose is essentially all gone and only xylose remains, aeration rate should be high. When the xylose is nearing exhaustion and its concentration is low, the aeration rate is reduced accordingly. Hence there is in batch fermentation an aeration regime of three phases, low, high, low. The figures given in Section 5, 0.15 vvm for 12 - 16 h, 0.25 - 0.30 for 16 - 24 h then 0.07 - 0.10 until fermentaion is complete, cannot be regarded as rigid, the proper aeration regime being dependent on the total compositon of the medium, on relative volumes of medium and fermenter, on agitation rate, temperature, yeast strain and probably nutrients and inhibitors. The first figure, 0.15 vvm, given in Section 5 is probably too high in most cases, and is based on furthering the low rate of xylose fermentation while the glucose is fermenting. It is probably better to set this at 0.05 - 0.10 vvm for the first phase. The figures given for the second and third phases are correct.

In the whole range of variables studied in this research project, aeration ranks as of great importance, and it is a subject we are studying further. However, the results of such studies can be generalized only with great caution: they cannot apply to all substrates, all media and all equipment configurations.

15.2 Acidity.

The proper pH for R yeasts has not been finalized. As stated in Section 5, successful pH ranges have been observed at 4.0 - 6.5, and we generally start fermentations at pH 5.5. We have not used buffers to control pH when fermenting with R yeast or with common bakers' yeast, although buffers are desirable with Zymomonas mobilis. We now suspect that corn steep liquor when used as a nutrient also may have a useful buffering effect. In addition, there is some feeling that pH 5.5 may be too low in the presence of marginally high concentrations of acetic acid, in the range of 0.4 - 0.6 g/L, and we are not aware of any significant disadvantage of using pH 7.0. This is a subject to be further explored.

15.3 Temperature.

Temperature of fermentation of prehydrolysates and acid hydrolysates of aspen and pine has been kept at 30 - 32°C, since this is known to be an optimal temperature range for most yeasts. However, it may well be that higher temperatures would give better performance with our R yeasts. In our experience, simultaneous saccharification and fermentation requires some temperature between the 45 - 55°C optimal for saccharification, and 30 - 32°C optimal for fermentation, and we have repeatedly found 37°C to be an excellent compromise. The rate-limiting factor is, of course, the saccharification rate, so we have not truly compared 37°C and 31°C fermentation rates. Also we have a statement from CBS Delft that our yeasts ferment well ar 41°C. This is an important finding which remains to be explored.

15.4 Literature.

We have not cited early literature in this review because to this date other laboratories have not reported results from the use of R yeasts in xylose fermentation. Early papers by Tom Jeffries, of the Forest Products Laboratory at Madison, and the TVA group at Muscle Shoals (Beck and Strickland) used culture collection yeasts which do not perform nearly as well as the R yeasts. The earliest papers describing conditions for successful xylose fermentation, that is successful in today's terms, are (i) Wayman and Tsuyuki "Fermentation of xylose to ethanol by Candida shehatae," Biotech. Bioeng. Symp. 15, 167 - 177 (1985) which has valuable data on the effects of cell concentration, oxygen concentration and other process variables for pure xylose solutions; (ii) Yu, Wayman and Parekh "Fermentation to ethanol of pentose-containing spent sulphite liquor," Biotech. Bioeng. 29, 1144 - 1150 (1987) which is the first reported adaptation of yeast to the stressful environment of this type of wood hydrolysate; and (iii) Parekh, Yu and Wayman "Adaptation of *Candida shehatae* and *Pichia stipitis* to wood hydrolysates for enhanced ethanol production," Appl. Microbiol. Biotechnol. **25**, 300 - 304 (1986), which describes clearly the adaptation procedure and its effects. More recent papers, such as nearly all of the 12 papers bound with this Report, elaborate on these themes.

We have in preparation other papers describing these R yeasts, and as soon as these are published the R yeasts will be deposited in public culture collections such as CBS in Delft, ATCC in Washington and NCYC in Norwich. We hope the general availability of these yeasts will stimulate other research workers to use them and report their findings.

16. Commercial nutrients.

In the fermentations carried out under this contract we have used as sources of nitrogen the commercial fertilizers diammonium phosphate and urea at low levels, 0.1 and 0.2 g/L respectively. This was enough to provide all the needed nitrogen and phosphate. In more recent work we have tested corn steep liquor as a source of nitrogen. Corn steep liquor contains a full complement of nutrients including phosphate and vitamins, but it needs to be used in much larger quantity, 5 g/L dissolved solids. Since corn steep liquor or its equivalent is available in considerable quantity in any fuel ethanol plant based on grain, or integrated with a grain processing plant, it is attractive as a source of nutrients.

Corn steep liquor is likely to vary in concentration and composition. There is not available as yet any firm data for characterization of corn steep liquor; nitrogen content and total solids analysis should serve to distinguish different batches until a simple nutritional test is established.

As reported in Section 5, it is essential that corn steep liquor be sterilized. The simplest way to sterilize it on a large scale would be to pass it through a St. Lawrence reactor at the proper time-temperature condition. In addition, the optimal quantity is not yet determined. Our work shows 5 g/L dissolved solids of our particular corn steep liquor to be satisfactory. The use of such a large amount of corn steep liquor may have the advantage of buffering the fermentation, preventing pH from falling to dangerous levels. However, too much corn steep liquor results in diversion of sugar from ethanol production to biomass production, thereby lowering ethanol yields. On the other hand, too little nitrogen also restricts alcohol production. When a source of corn steep liquor has been identified and characterized, the best quantity to use can readily be determined in shake flasks.

The corn steep liquor we used came from Casco, Cardinal, Ontario, and St. Lawrence or other corn steep liquors may have somewhat different nutritional qualities. However, unless something comes up of which we are not yet aware, we plan to use corn steep liquor as sole nutrient in our laboratory fermentations, and would so recommend to others.

17. Process design: conditions for industrial fermentation of aspen and pine hydrolysis process streams.

Since there are no industrial fermentations of aspen and pine hydrolysis process streams, we ask that our suggestions, made in response to a specific request by Bio-Hol, be regarded as educated speculation based on laboratory experience and relevant industrial operations. We shall here address questions of inhibition by and removal of acetic acid, separate fermentation of prehydrolysates and hydrolysates, xylose recovery, lignin recovery, nutrients, aeration and other operating conditions such as pH, temperature and cell concentration.

The general process design is assumed to be prehydrolysis, separation of hemicellulose sugar solutions, hydrolysis of the cellulose and recovery of the glucose so formed, leaving a residue mainly lignin. Thus there are two fermentable streams, the hemicellulose sugars mainly xylose, and glucose. Prehydrolysis using a mild acid catalyst results in a high proportion of hemicellulose sugars as monomers rather than oligomers found when only steam is used in prehydrolysis. The Wenger cooker-extruder is quite satisfactory for that purpose, as also the Masonite gun, the Sunds Defibrator, and the Stake reactor. The choice among these depends entirely upon common engineering criteria. Such criteria apply also to equipment for separation of the hemicellulose solution. For acid hydrolysis, the St. Lawrence continuous high shear plug flow reactor is considered the equipment of choice.

Fermentation process design may be either batch fermentation with cell recycle; batch fermentation in the St. Lawrence style which largely conserves active yeast; cascade continuous fermentation with cell recycle; or immobilized cell continuous fermentation. Each of these has its advantages and disadvantages: for a first plant either the St. Lawrence process lor the cascade process, which operates very successfully on spent sulphite liquor at the Bellingham plant of Georgia Pacific, would seem advisable.

17.1 Acetic acid and other inhibitors.

Hemicellulose sugar solutions obtained in prehydrolysis contain acetic acid, which, while a nutrient at very low concentrations, is a strong fermentation inhibitor at concentrations of 0.5+g/L (NOT percent). Aspen prehydrolysates are particularly rich in acetic acid, about 8 - 10 g/L, while pine prehydrolysates have about 3 - 5 g/L. The concentration of acetic acid needs to be reduced, either by dilution where that is acceptable, or by removal. For acetic acid removal two methods commend themselves, vapour distillation and ion exchange. Vapour distillation of acetic acid from hemicellulose solutions is rapid and effective. It should be stressed, however, that evaporation of hemicellulose solutions for the purpose of concentrating the sugars is only effective in removing acetic acid if the pressure is atmospheric or above. Vacuum evaporation only concentrates the acetic acid, and does not remove it. We have no experience with ion exchange removal of acetic acid from hemicellulose solutions, but there is no a priori reason for doubting its usefulness.

In process design it is necessary to provide for acetic acid removal, and the best method we know is vapour distillation.

Hemicellulose solutions contain other inhibitors such as those derived from lignin, or furfural. These have the effect of slowing fermentation, particularly xylose fermentation. Furfural is largely removed in vapour distillation. The yeasts are to a large extent adaptable to these inhibitors. The best policy, however, is to choose prehydrolysis conditions in which the formation of such inhibitors is minimal.

17.2 Separate fermentation of prehydrolysates and hydrolysates.

The use of R yeasts for fermentation has made it unnecessary to ferment prehydrolysates and hydrolysates separately. These yeasts ferment the mixture of wood xylose and glucose comfortably.

We have found that the fermentation of hydrolysates, that is the glucose component of the wood, proceeds with far less difficulty than the prehydrolysates, being rapid and efficient with R yeasts, common bakers' yeast and *Zymomonas mobilis*. Thus the option of separate fermentation remains. If the hemicellulose solution is to be used for some purpose other than fermentation, then either common bakers' yeast or *Z. mobilis* should be used for fermentation of the hydrolysates. The well-known cautions about the bacterium need to be weighed against its advantages over yeast.

17.3 Xylose recovery.

The Finnish Sugar Company uses Stake-generated birch prehydrolysates for recovery of xylose, which is then hydrogenated to xylitol, a sweetener which enjoys a good market. The xylose of prehydrolysis may also be converted to furfural, as is done with agricultural byproducts in the QO Chemical plant in West Belle Glade, Florida. These are two obvious examples of upgrading xylose to higher valued products. They would be particularly relevant to aspen, less so for pine. The separate recovery of xylose from aspen prehydrolysates and its conversion to higher valued products is a relatively major undertaking, and in assessing its feasibility, possible competition from agricultural residues as feedstock needs to be taken into account.

17.4 Lignin recovery.

In the assumed process design, lignin remains after hydrolysis, contaminated with some proportion of cellulosic residue and other matter. If used as fuel, these contaminants do not matter. If lignin is to be used for some other purpose, such as vanillin production, it may have to be purified, perhaps via sulphonation for vanillin, or demethylation for panelboard resins. In any industrial wood to ethanol plant, the quantity of lignin generated will be great, 20 to 30 % of the wood substance, and lignin will need to be used or disposed of. For use as fuel, free acid must first be neutralized and the water content reduced, which adds to the cost. The net value is not great. Lignin products are used in North America at the rate of 1400 tonnes per day, so there may be room for entry into this market.

Lignin may be recovered after prehydrolysis by extraction with NaOH at moderate temperature (70 - 90°C) or by solution in ethanol at 150 - 160°C in pressure vessels. Recovery of lignin by NaOH extraction is incomplete, only 25 - 50% of the lignin going into solution, while ethanol extraction is essentially quantitative. This step may become desirable if excessive fouling of the St. Lawrence reactor is experienced.

High volume uses for lignin have been sought intensively in many research centres for many years, so far without encouraging results. Lignin sales on a large scale would be a risky income component upon which to base the feasibility of an ethanol plant. Chicken feed binders are among the higher volume uses. We see it also as a food additive because of its effect in lowering blood cholesterol.

17.5 Nutrients.

Nitrogen may be supplied to fermentation by ammonia to the extent that it serves also to neutralize residual acid. Common fertilizers diammonium phosphate and urea are also, usually in combination, excellent sources of low cost nitrogen at very low concentrations, 0.1 - 0.2 g/L. Corn steep liquor as a source of nitrogen has the advantage that it contains vitamins and other yeast growth stimulants, but the quantities required are much larger than the fertilizers, being about 5 g dry solids per litre. In addition, corn steep liquor is frequently contaminated with acid-loving lactic acid bacteria, which spoil fermentation, and it needs to be sterilized.

We recommend neutralization with ammonia and the addition of sterile corn steep liquor to provide the rest of the needed nitrogen. Then that is all the nutrient required.

17.6 Aeration.

In separate fermentation of hydrolysate, little or no aeration is required. For separate fermentation of prehydrolysates or mixed prehydrolysate-hydrolysate, an aeration regime needs to be established. In continuous cascade fermentation with at least three fermenters in series, the first fermenter requires very little air, perhaps 0.05 vvm, the second requires more air, 0.20 - 0.25 vvm, and the third much less, about 0.1 vvm. In batch fermentation, aeration needs to follow a time-related cycle in imitation of that, that is about 0.05 vvm for the first 12 - 24 h, then 0.20 - 0.25 vvm for an additional 12 - 24 h, then about 0.1 vvm to the end of fermentation.

These figures are a good place to start. The optimal aeration regime will depend upon many factors including fermenter configuration, yeast or mixed yeast culture employed, substrate composition including inhibitors, and others, and will require adjustment with each change of feedstock, temperature, pH or other fermentation conditions, and cannot now be predicted accurately in advance.

17.7 Temperature, pH and cell concentration.

Fermentation temperature of 30 - 32°C is common and satisfactory. With the R yeasts, higher temperatures may be more efficient. The best temperature for the R yeasts has not been established. Pending its establishment, a plant designed to use R yeasts could well begin operations at 30 -32°C and raise temperature gradually, say 1°C per month while monitoring efficiency, a form of operational research which could pay dividends, and we would so recommend.

The best pH to start would be 5.5. There may be some advantage in going to higher pH in that the yeast may tolerate more acetic acid at higher pH.

Higher cell concentrations are advantageous, the upper limit being determined only by rheological considerations. High cell concentrations need to be achieved by either cell recycling, or by the St. Lawrence fermentation method. Recycling is very important in terms of adaptation to inhibitors in the medium, and in achieving rapid, efficient fermentation.

PART II Experimental methods and data

1. Materials and methods

1.1 Microorganisms

The yeast we have found most suitable for xylose fermentation is *Pichia stipitis* CBS 5776. The key to the successful fermentation of any solution containing xylose is, however, in the generation of an adapted strain of this yeast. Acclimatization under stress results in the generation of a new breed of the yeast which is efficient in its ability to ferment such solutions, and robust in its ability to withstand inhibitors. Such an acclimatized yeast is labelled *Pichia stipitis* R.

The procedure for adaptation follows. A stock culture of *P. stipitis* is maintained on a YMP slant and transferred every two months. The purity of the culture is checked before each transfer. The composition of the medium is as follows:

yeast extract	3 g/L
malt extract	3 g/L
peptone	5 g/L
xylose	20 g/L
pH	5.4
agar	30 g/L

A loopful of the stock culture is first grown in 50 mL of YMP medium having 20 g/L xylose in a 125 mL conical flask with a foam stopper. The culture is grown at 30 C for 24 hours on a rotary shaker at 150 rpm. The growth so obtained is inoculated at a 2% rate into a similar flask having YMP nutrients but the xylose is replaced by the wood sugar solutions of interest. The concentration is usually 40 g/L total sugars. The acetic acid content should be below 0.5 g/L. Moreover, since the wood hydrolysate or prehydrolysate will be acidic, the pH of these liquors must be raised to 5.5 with lime, prior to the inoculation. The wood sugar solution need not be sterilized. The inoculated flask is then incubated for 24 hours at 30 C on the rotary shaker. This represents the first cycle of adaptation. The growth obtained from this first cycle of adaptation is then inoculated into a fresh flask with the same composition, and the procedure of acclimatization and recycling the yeast in the wood solution is repeated at least 10 times. After 10 or more cycles of repeated growth on the wood hydrolysate the yeast is well adapted to the sugar solution of interest. The end result is the evolution of the culture known as the R strain or the recycled strain of *P. stipitis*. This culture is isolated on a YMP agar plate having 2% xylose. The purity of the culture is rechecked and it is transfered to a xylose-YMP slant. *P. stipitis R* is diploid, and the cells appear larger in size than the parent strain.

One of the characteristics of P. stipitis R is its ability to adapt to various liquors derived from lignocellulosic hydrolysis. In order to ferment liquors having xylose, the yeast can be adapted to that particular solution and then fermented. We have also noticed that the robust nature of this yeast can be maintained if it is regularly recycled in various liquors rich in xylose.

All fermentations are performed with the R strain of the yeast P. stipitis. The inoculation yeast for large scale fermentation has to be cultured in the same medium as the fermentation broth but at lower sugar levels. The cells are centrifuged at 2000 rpm for 10 minutes, washed once with saline and the biomass concentration estimated gravimetrically. The cells are inoculated so that the fermentation medium normally contains 12 - 15 g dry cells weight/L of broth.

1.2. Nitrogen nutrients

Until recently, the nitrogen nutrients which we have employed in fermentation have been YMP having the following concentrations of the ingredients:

yeast extract	1 g/L
malt extract	1 g/L
peptone	2 g/L
pH	5.4

We have also noticed that diammonium phosphate and urea at 0.1 and 0.2 g/L give satisfactory results, but this depends on the feedstock being fermented.

1.3. Aeration

Xylose fermentation is strictly regulated by the aeration rate supplied. Higher rates result in biomass buildup whereas suboptimal rates result in slow consumption of xylose and its transformation to ethanol. Xylose fermentation by P. stipitis R is not aerobic nor anaerobic, but a compromise known as "semiaerobic." The aeration requirement in wood sugar fermentation is further influenced by the concentration of the sugars and the biomass in the fermenter. When total hydrolysate is employed, initially a low rate of aeration, 0.15 vvm, is adequate for the anaerobic fermentation of the glucose component of the hydrolysate. For residual xylose fermentation, the aeration rate has to be fine tuned, in some cases even raised to 0.25 vvm so that it remains optimal for xylose assimialtion and at the same time minimal for ethanol respiration. Also monitoring of the sugar levels is important when regulating the air flow, since in the absence of substrate and the presence of high biomass and air, it can result in consumption of the accumulated ethanol.

1.4. Temperature

Temperature should be maintained at 30 ± 2 C throughout.

1.5. Antifoam

Antifoam 10 - 20 ppm polypropylene glycol.

1.6. Analysis

Ethanol was determined by gas chromatography using n-propanol as the internal standard. Sugars in the culture supernatant were analysed by Waters Sugar Analyzer No. 1 HPLC with an Aminex HPX 87P column (BioRad) and by eluting with water at 65 C flow rate 0.5 mL/minute, and Waters RI detector. Yeast cell dry weight g/L was determined by gravimetric methods and optical density at 660 nm using a Spectronic 20 and converted to g/L established by drying the washed cells to a constant weight in a freeze drier.

1.7. Yield calculation

Theoretically 100 g glucose will yield 51 g ethanol, and 100 g xylose 51 g ethanol. On an average basis we assume that for every 100 g total sugars fermented, glucose and xylose, a yield of 47 g ethanol will be possible. In normal practice, 100 % efficiency is never achieved: there are losses due in part to sugars being consumed for biomass synthesis, by-product synthesis (acids, xylitol, glycerol) and maintenance-related reactions. A 90% yield, about 44 g ethanol/100g sugar fermented, is considered satisfactory.

1.8. Preparation of inoculum

Adjust for different volumes.

To be combined:

3 g/L yeast extract
3 g/L malt extract
5 g/L peptone,
mixed in 1/2 volume required as final volume,

28 g/L glucose
12 g/L xylose,
mixed in 1/2 volume required as final volume.

Adjust each flask to pH 5.5 using NaOH or HCl, 10% HCl may be needed for peptone mixture. Stopper flasks and place 1in autoclave on slow exhaust, 121 C, for 15 minutes. jCool to room temperature and combine in sterile flask. Inoculate with yeast, 2 - 3 loopfuls. Again stopper flask and shake at 30 C and 150 rpm for 3 - 4 days.

Place inoculum in centrifuge bottles (sterile) and balance. Do under aseptic conditions. Centrifuge at 2000 rpm for 10 minutes. Pour off supernatant and discard. Add enough saline* (sterile) to loosen pellet and transfer to a graduated cylinder (sterile) to record volume V.

*Saline solution: 8.25 g NaCl/L DDH2O

Determination of NOD: Dilute 1 mL of inoculum from cylinder in 100 mL H2O. Read the transmittance on the spectrophotometer at 660 nm. Use distilled water as the blank.

OD = -log (T) x 1000
NOD = corrected value based on OD and obtained from charts.

$$g/L = \frac{NOD - 2.8}{1010} \times DF\ (= 100)$$

$$g = g/L \times V$$

Addition of inoculum to sample for fermentation:

In a sterile flask add a known volume of sample to be fermented, = mL. Inoculum load is, ideally, over 10 g/L. Generally we exceed this amount, to guarantee performance, and add 15 g/L. Raise pH of sample in flask to 5.5 with lime. To calculate g of inoculum needed:

$$\frac{15\ g}{1000\ mL} = \frac{y\ g}{x\ mL}$$

$$y\ g = \frac{15\ x}{1000}$$

To calculate what this is in terms of inoculum volume:

$$z\ mL = \frac{(x)\ (V)}{y}$$

In sterile centrifuge bottles, place desired volume of inoculum. Add sterile saline. Centrifuge at 2000 rpm for 10 minutes. Pour off supernatant and discard. To sample add 0.2 g/L urea and 0.1 g/L DAP. Add small amount of sample-to-be-fermented to bottle and dissolve inoculum pellet. Transfer back to fermentation flask.

Stock solution, weigh out:
200 mg urea (0.2 g)
100 mg DAP (0.1 g)

Place in glass test tube. Add 10 mL of sterile saline solution using sterilized 10 mL pipet. Seal and dissolve. When using for fermentation, add 1 mL for every 100 mL of sample for fermentation.

Sampling:

Always remember to take sample at t = 0. Take the sample after the inoculum has been added and refrigerate immediately to prevent fermentation. Continue to take samples at various times. Always take two samples, one for TRS estimation and one for alcohol estimation.

1.9. Fermentation procedures

For more detail on fermentation procedures, see Part I, pages 12 - 14, which has further information about xylose fermentation, particularly the effects of nutrients, pH, cell conentration, air flow, inhibitions and yield calculations, and these details need not be repeated here.

2. Fermentation data, tables and graphs

2.1 HPLC curves.

In Part I pages 14 - 16 are given a large number of examples of fermentation of solutions of xylose, glucose-xylose mixtures, prehydrolysates and mixed prehydrolysates-hydrolysates, as well as references to where more detail can be had in the Monthly Progress Reports submitted to Bio-Hol. Following here are primary HPLC sets of curves for two important runs.

Run D-1, D-2

Fermentation of steam stripped aspen prehydrolysate in the 28 L fermenter at Bio-Hol with *P. stipitis* R:

At t = 0	glucose = 16.1 g/L	xylose = 40.5 g/L
24 h	3.3	37.6
50	0	12.7
64	0	5.6

At 64 h, all glucose and 86.2% of the xylose was fermented. These results show clearly the diauxie effect we experience in the larger scale runs. This does not show up in shake flask runs.

Run D-3, D-4

Fermentation of mixed aspen prehydrolysate and hydrolysate in the 28 L fermenter at Bio-Hol with *P. stipitis* R:

At t = 0 glucose = 73.9 g/L xylose = 28.6 g/L
28 h 1.6 31.8
48 1.1 14.9
64 1.5 5.6

At 64 hours, 98% of the glucose and 80.4% of the xylose was fermented. Again, no fermentation of the xylose was observed until after the glucose was fermented. The production of alcohol in this run was as follows (data on sheets D-5 and D-6):

At t = 18.5 ethanol = 30.7 g/L
21 34.6
39 41.5
50 44.5
64 46.7

Based on glucose + xylose, 0.49 g ethanol/g sugar consumed. Based on Total Reducing Sugars, 0.46 g ethanol/g sugar consumed.

These slow rates of fermentation are attributable first to the diauxie effect, and then to improper aeration control. We still have a lot to learn about the relationship of air flow to rates and efficiency of xylose fermentation.

The following data relate to fermentation of HCl-hydrolysed aspen cellulose hydrolysate, made in the SLR at Bio-Hol, fermented in the 4 L fermenter at U of T using common yeast instead of R yeast (see sheet D-7):

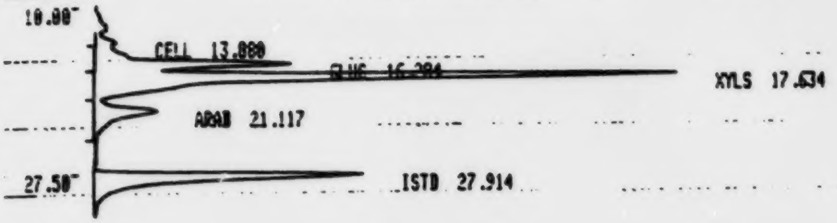
At t = 0 glucose = 53.5 g/L xylose = 5.1 g/L
30 h 1.4 5.8 ethanol = 22.9 g/L

Thus at 30 h, 97.4% of the glucose was fermented, but none of the xylose. Ethanol produced at 2.9% by volume was 0.44 g/g sugar consumed. Upon receipt, this hydrolysate was a dark chocolate brown and of very high viscosity. We had small hope for it. However, upon adding nutrients and yeast, the small dilution so experienced lowered the viscosity significantly, and the fermentation went smoothly and generally satisfactorily.

Pine hydrolysate, also HCl-catalysed in the SLR reactor, fermented even more easily, fermentation being complete in 12 h, 35.2 g/L ethanol (4.5% by volume) being 0.49 g ethanol/g glucose consumed.

HPLC analysis at 4 g l⁻¹ aspen - prehydrolysate fermentation 28C at Biohol C' Y-1 (p 38)

```
SAMPLE   3   ———      11:50  NOV. 12 1899
```



```
CAL. METHOD    03
                SF            PA            PB
        .100000E+01  .100000E+01  .160000E+02

ISTD PEAK       27.914      8548019

NO.     NAME        RT      A OR H    MK         CONC

 1      CELL      13.880     632461    M        0.6812
 2      GLUC      16.284    6472336    M        7.6610
 3      XYLS      17.634   16753785    M       19.3089
 4      GALA
 5      ARAB      21.117    2890316    M        1.0745
 6      MANN
 7      ISTD      27.914    8548019            16.0000

        TOTAL               35296918            44.7257
```

TRS = 60gl⁻¹

T=0

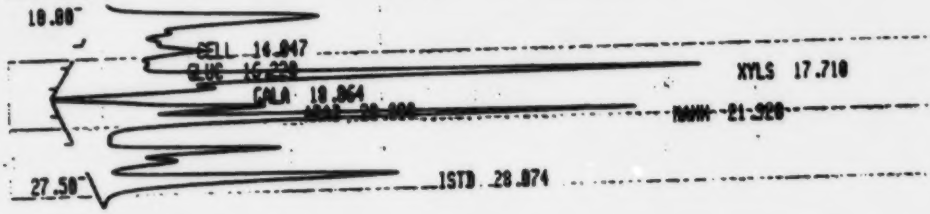
Biohol

DF

1:2·1

TRS 60gl⁻¹

xylose = 40.5g/l



```
CAL. METHOD    03
                SF            PA            PB
        .100000E+01  .100000E+01  .160000E+02

ISTD PEAK       28.074      9807736

NO.     NAME        RT      A OR H    MK         CONC
                                                 1.4776
 1      CELL      14.047    1573435    M         1.6474
 2      GLUC      16.220    1678978    M        18.7970
 3      XYLS      17.710   18543680    M         3.0574
 4      GALA      18.864    3629295              2.0049
 5      ARAB      20.880    6223059    M         9.8099
 6      MANN      21.920   15106485    M        16.0000
 7      ISTD      28.074    9807736

        TOTAL               56562668            52.7944
```

T=24.

DF=2

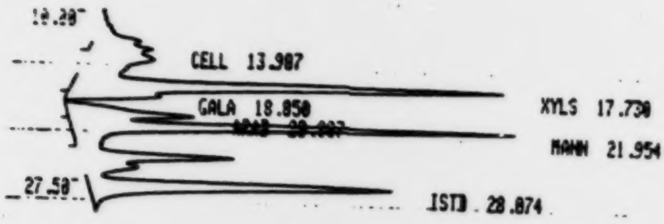
=

30gl⁻¹ xylose

xylose

tB1 D-2
(p 39)

HPLC analyses on the progress of Aspen wood
[illegible]hydrolysate fermentation.



T= 50

```
CAL. METHOD    03
             SF              PA              PB
     .100000⏨+01   .100000⏨+01   .160000⏨+02

ISTD PEAK       28.074      9442882

NO.     NAME       RT      A OR H    MK       CONC

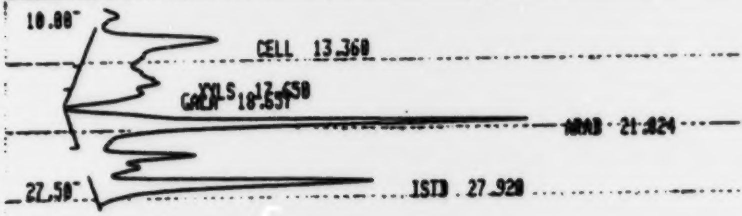
  1     CELL    13.987    1588364     M      1.5493
  2     GLUC
  3     XYLS    17.730   12081419     M     12.7196
  4     GALA    18.850    1994878             1.7454
  5     ARAB    20.887    4032605     M      1.3494
  6     MANN    21.954   11376143     M      2.6729
  7     ISTD    28.074    9442882            16.0000

       TOTAL             40516292            41.0368
```

DF=1

≈ 12.7 gl^{-1}

```
           SAMPLE  14                   15:14  NOV. 09 1988
```



```
CAL. METHOD    03
             SF              PA              PB
     .100000⏨+01   .100000⏨+01   .160000⏨+02

ISTD PEAK       27.920      9477177

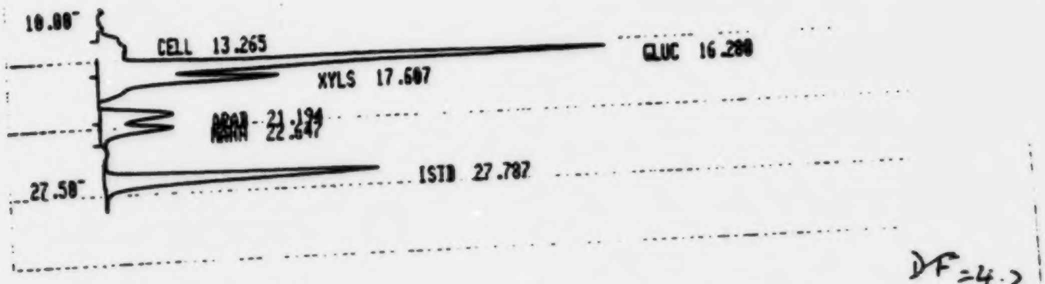
NO.     NAME       RT      A OR H    MK       CONC

  1     CELL    13.360    8679048     M      8.4315
  2     GLUC
  3     XYLS    17.650    5433051     M      5.6477
  4     GALA    18.657    3111332             2.4499
  5     ARAB    21.824   15427578     M      5.1732
  6     MANN
  7     ISTD    27.920    9477177            16.0000

       TOTAL             42128186            37.7025
```

7.64
6 gl^{-1}

HPLC profiles of the progress of Aspen wood (prehydrolysate + hydrolysate) fermentation – U of T ① D-3 (P40)



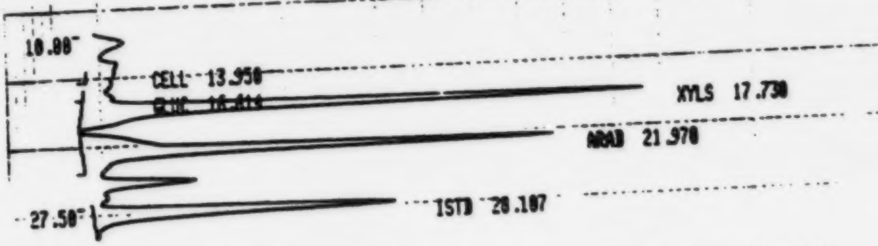
DF=4.3

```
CAL . METHOD    03
                   SF                 PA                 PB
          .100000E+01  .100000E+01  .160000E+02

ISTD PEAK        27.787      8414756
                                                          CONC
NO.      NAME        RT        A OR H      MK
                                                         0.6047
  1      CELL     13.265       552750       M           17.1768
  2      GLUC     16.280     14285377       M            6.6456
  3      XYLS     17.607      5676311
  4      GALA                                            1.0027
  5      ARAB     21.194      2655021       M            1.7154
  6      MANN     22.647      2266470       M           16.0000
  7      ISTD     27.787      8414756
                                                        43.1453
                              33850686
         TOTAL
```

T=0

– 11 g l⁻¹



50

```
CAL . METHOD    03
                   SF                 PA                 PB
          .100000E+01  .100000E+01  .160000E+02

ISTD PEAK        28.107      9129362
                                                          CONC
NO.      NAME        RT        A OR H      MK
                                                         0.9618
  1      CELL     13.950      1031293       M            0.8245
  2      GLUC     16.614       845148       M           15.8962
  3      XYLS     17.730     15541642
  4      GALA                                            4.4683
  5      ARAB     21.970     13833890       M
  6      MANN                                           16.0000
  7      ISTD     28.107      9129362
                                                        38.1510
                              40381336
         TOTAL
```

DF=2.0

T=25

31.7 g l⁻¹

HPLC profiles of the progress D [illegible]

(p41)

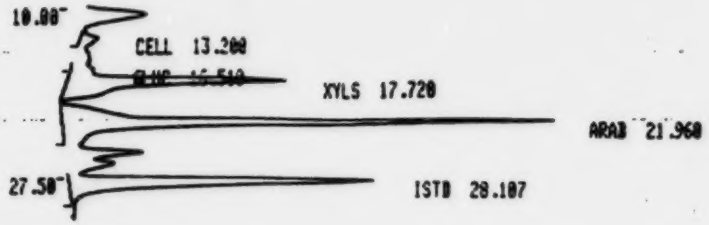
D-4

```
SAMPLE  17                15:16  OCT. 02 1988
```

2.05 DF

2.1 DF

T=48



```
CAL. METHOD   03
        SF              PA              PB
  .100000E+01   .100000E+01   .160000E+02

ISTD PEAK      28.107      9268998      M

NO.     NAME        RT       A OR H     MK         CONC

 1      CELL     13.200      577757      M       0.5307
 2      GLUC     16.510      573689      M       0.5513
 3      XYLS     17.720     7228232               7.2817
 4      GALA
 5      ARAB     21.960    13931753      M       4.4321
 6      MANN
 7      ISTD     28.107     9268998      M      16.0000

      TOTAL                31580430              28.7959
```

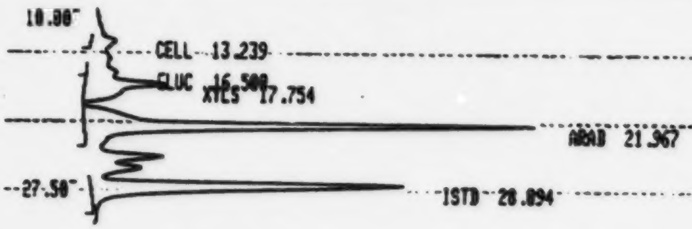
49

DF=2.1

~15 gl^{-1}

Xylose

```
SAMPLE  16                    14:43  OCT. 02 1988
```



```
CAL. METHOD   03
        SF              PA              PB
  .100000E+01   .100000E+01   .160000E+02

ISTD PEAK      28.094      9645022      M

NO.     NAME        RT       A OR H     MK         CONC

 1      CELL     13.239      926781      M       0.8181
 2      GLUC     16.500     1042062      M       0.9623
 3      XYLS     17.754     3616208               3.5009
 4      GALA
 5      ARAB     21.967    12628961      M       3.8610
 6      MANN
 7      ISTD     28.094     9645022      M      16.0000

      TOTAL                27859035              25.1425
```

T= 64

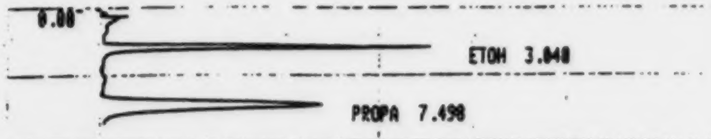
DF

1.6

~8 gl^{-1}

Ethanol profiles during the fermentation of prehydrolysate + hydrolysate mixture ~~[illegible]~~ Bichol (28L).

[1] D-5 (p 42)



T=18.5
DF→4.8

289

```
CAL. METHOD    03
              SF          RA          PB
   .100000E+01  .100000E+01  .700000E+01

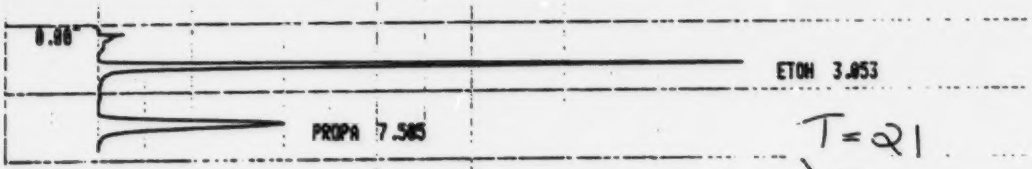
ISTD PEAK        7.498       163252

NO    NAME       RT       A OR H    MK        CONC

 1    ETOH      3.048     110620             6.2230
 2    PROPA     7.498     163252             6.9999

      TOTAL               273873            13.2230
```

30 gl^{-1}



T=21
DF→7.5

```
CAL. METHOD    03
              SF          RA          PB
   .100000E+01  .100000E+01  .700000E+01

ISTD PEAK        7.505       138024

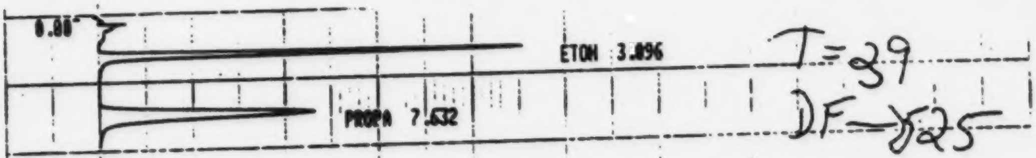
NO.   NAME       RT       A OR H    MK        CONC

 1    ETOH      3.053     208402            13.8667
 2    PROPA     7.505     138024             6.9999

      TOTAL               346427            20.8667
```

290

gl^{-1} 34.6



T=29
DF→~~5~~25

DF 5.25

```
CAL. METHOD    03
              SF          RA          PB
   .100000E+01  .100000E+01  .700000E+01

ISTD PEAK        7.632       163571

NO.   NAME       RT       A OR H    MK        CONC

 1    ETOH      3.096     140613             7.8948
 2    PROPA     7.632     163571             6.9999

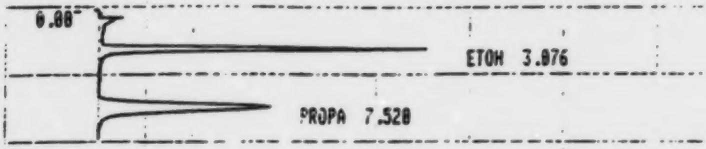
      TOTAL               304185            14.8948
```

gl^{-1} =41.5

[illegible]

Aspen wood mixture fermentation. (28L) [2]

D-6
P43



T = 50
DF → 6

```
CAL. METHOD    03
              SF              PA              PB
       .100000E+01  .100000E+01  .700000E+01

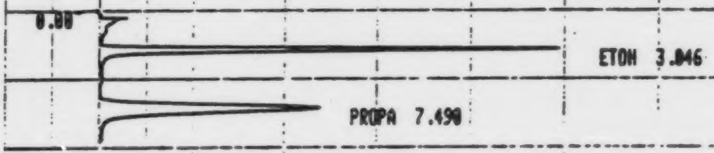
ISTD PEAK        7.520       129093

NO.    NAME          RT      A OR H    MK          CONC

 1     ETOH        3.076     106909                7.6057
 2     PROPA       7.520     129093                6.9999

       TOTAL                 236003               14.6057
```

= 44.5 gl^{-}



T = 64
DF → 5.5

```
CAL. METHOD    03
              SF              PA              PB
       .100000E+01  .100000E+01  .700000E+01

ISTD PEAK        7.490       166651

NO.    NAME          RT      A OR H    MK          CONC

 1     ETOH        3.046      54828                8.5323
 2     PROPA       7.490     166651                6.9999

       TOTAL                 321479               15.5323
```

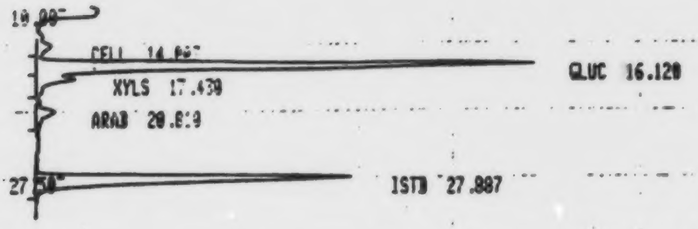
46.7 gl^{-}

HCl-catalysed Aspen hydrolysate from SCR plug flow reactor V-8
4L fermentation (U of T) S. cerevisiae

p 44

④

1:4 Dil



T=0

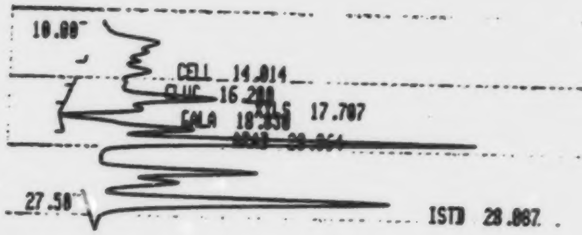
```
CAL. METHOD    03
                SF              PA              PB
       .100000E+01    .100000E+01    .160000E+02

ISTD PEAK        27.887     8747829      M

NO.     NAME       RT       A OR H     MK          CONC

 1      CELL     14.007      530591     M        0.5258     2 gl-1
 2      GLUC     16.120    12500701     M       13.3664     56 gl-1
~~ 3      XYLS     17.430     1108455     M        1.2700~~   ~ 5gl-1
 4      GALA
 5      ARAB     20.810      740127     M        0.7995   -  4gl-1
 6      MANN                                                 63gl-1
 7      ISTD     27.887     8747829     M       16.0000

        TOTAL              23627704             31.9617
```



T=30

DF=1

```
CAL. METHOD    03
                SF              PA              PB
       .100000E+01    .100000E+01    .160000E+02

ISTD PEAK        28.087     9760499

NO.     NAME       RT       A OR H     MK          CONC

 1      CELL     14.014     1063274     M        1.0033
 2      GLUC     16.200     1464072     M        1.4435   glucose
 3      XYLS     17.707     5651821     M        5.7567   ~ xylose
 4      GALA     18.830     2141185              1.8125
 5      ARAB     20.864     4218803     M        1.3657
 6      MANN
 7      ISTD     28.087     9760499             16.0000

        TOTAL              24299656             27.3820
```

2.2 *Zymomonas mobilis* fermentation of SLR-HCl hydrolysates

It is not too difficult to visualize circumstances where separate fermentation of the hydrolysates may be desirable, for example if crystalline xylose or furfural are to be products. This study compared *S. cerevisiae* and *Z. mobilis* is such fermentation.

Materials and methods

Prehydrolysis of aspen and of pine woods were carried out in the Wenger reactor. The hemicellulose sugars were washed from the prehydrolysed woods, and they were then subjected to second stage hydrolysis in the SLR plug flow reactor at Bio-Hol. The conditions were: aspen 220 C for 2.2 minutes, pH 1.5, acid 0.3%; pine 220 C for 1.6 minutes, pH 1.5, acid 0.28%. The hydrolysates so obtained were thick and viscous, very hard to handle. They were diluted 15 - 20% by volume on adding soluble nutrients, % by weight:yeast extract 1.0; KH_2PO_4 0.1; $MgSO_4.7H_2O$ 0.05; $(NH_4)_2SO_4$ 0.1; pH 5.5. They were then much easier to handle, and they were inoculated with cultures of *Z. mobilis*, 9.8 g/L, and *S. cerevisiae* 11.7 g/L.

Results and discussion

The results are shown in Table 1. Both organisms fermented these hydrolysates successfully, converting more than 94% of the glucose to ethanol. The *Z. mobilis* fermentation time was 4 h for pine and 7 h for aspen, while the *S. cerevisiae* required 10 and 16 h for complete fermentation. These are slower fermentations than we experienced with enzyme saccharified hydrolysates where *Z. mobilis* fermented all the glucose to ethanol in 15 minutes. This suggests that inhibitors in the acid hydrolysates slowed the fermentations, although the rates were still quite satisfactory.

Table 1 Fermentation of acid hydrolysates of pine and aspen by *Z. mobilis* and by *S. cerevisiae*

	TRS	glucose	residual TRS	ethanol g/L	yield g/g
aspen	51.38	49.9			
Z. mobilis			1.4	24.2	0.48
S. cerevisiae			1.0	23.8	.47
pine					
Z. mobilis			2.4	24.7	.49
S. cerevisiae			2.2	24.1	.48

2.3. Fermentation data, tables and graphs

TABLE/GRAPH No.	SUBSTRATE	FERMENTER
1	xylose	4 L
2	xylose	28 L
3	glucose-xylose	28 L
4	aspen prehydrolysate	28 L
5	aspen hydrolysate	4 L
6	aspen prehydrolysate + hydrolysate	28 L
7	pine prehydrolysate	4L
8	pine hydrolysate	4L
9	pine prehydrolysate + hydrolysate	4L

Discussion of Tables and Graphs begins on page 65.

Table No. 1
Experiment No. 1 Date April 7, 1988
Substrate xylose Medium, and medium preparation
2.7 L medium in 4 L fermenter, YMP @ 2, 2, 3 g/L
pH 5.0, 30°C, agitator 200 rpm, 12 ppm polypropylene glycol
yeast Pichia stipitis R

	t = 0	t = 24	t = 48	t = f (32h)
TRS g/L	28	9		1.5
fermentable sugars, g/L				
biomass g/L	1.4	5.5		6.7
ethanol g/L		8.7		13.0

aeration vvm t = 0, 0.33; t = 10, 0.19; t = 24, 0.07

fermentation time t = f 32h
biomass OD, t = 0, 0.15 ; t = f, 0.065: at t = 0, 1/10; t = 32, 1/100
" log OD, t = 0 0.82 ; t = f 1.07
residual glucose (t = f) g/L 0
" xylose (t = f) g/L 1.5
" mannose (t = f) g/L 0

Yx/s	g biomass/g sugar consumed	0.19
Yp/s	g ethanol/g sugar consumed	.485
Vp	g ethanol/L-h	.40
Qp	g ethanol/g biomass(f)-h	.076

GRAPH # 1

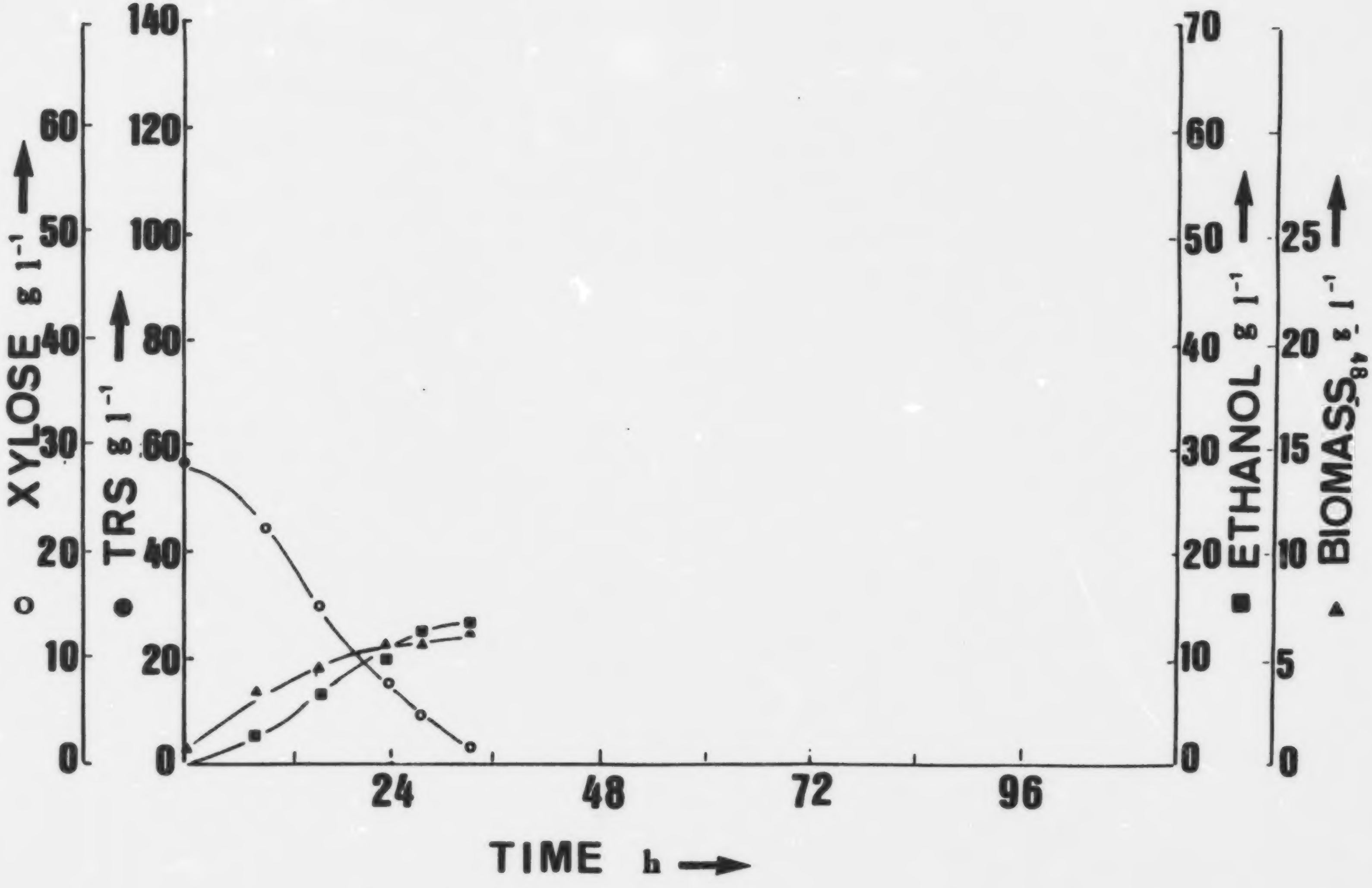

○ XYLOSE g l⁻¹
● TRS g l⁻¹
■ ETHANOL g l⁻¹
▲ BIOMASS, g l⁻¹
0
10
20
30
40
50
60
0
20
40
60
80
100
120
140
0
10
20
30
40
50
60
70
0
5
10
15
20
25
24
48
72
96
TIME h

Table No. 2
Experiment No. 2 Date June 13, 1988
Substrate xylose Medium and medium preparation
17 L in 28 L fermenter, YMP @ 2,2,3 g/L, pH 5.2
30°C, agitator 200 rpm, 15 ppm polypropylene glycol
yeast *Pichia stipitis* R

	t = 0	t = 24	t = 48	t = f (64h)
TRS g/L	56.6	30.6	7.6	3.2
fermentable sugars, g/L				
biomass g/L	2.8	10.0	11.4	13.2
ethanol g/L		10.2	22.0	23.4
aeration vvm	0.24	0.12	0.08	

fermentation time t = f 64 h
biomass OD, t = 0,0.032; t = f,0.145, both 1/100
" log OD, t = 0,-1.49; t = f, -0.838

residual glucose (t = f) g/L	0
" xylose (t = f) g/L	3.2
" mannose (t = f) g/L	0

Yx/s	g biomass/g sugar consumed	0.24
Yp/s	g ethanol/g sugar consumed	.44
Vp	g ethanol/L-h	.37
Qp	g ethanol/g biomass(f)-h	.035

GRAPH # 2

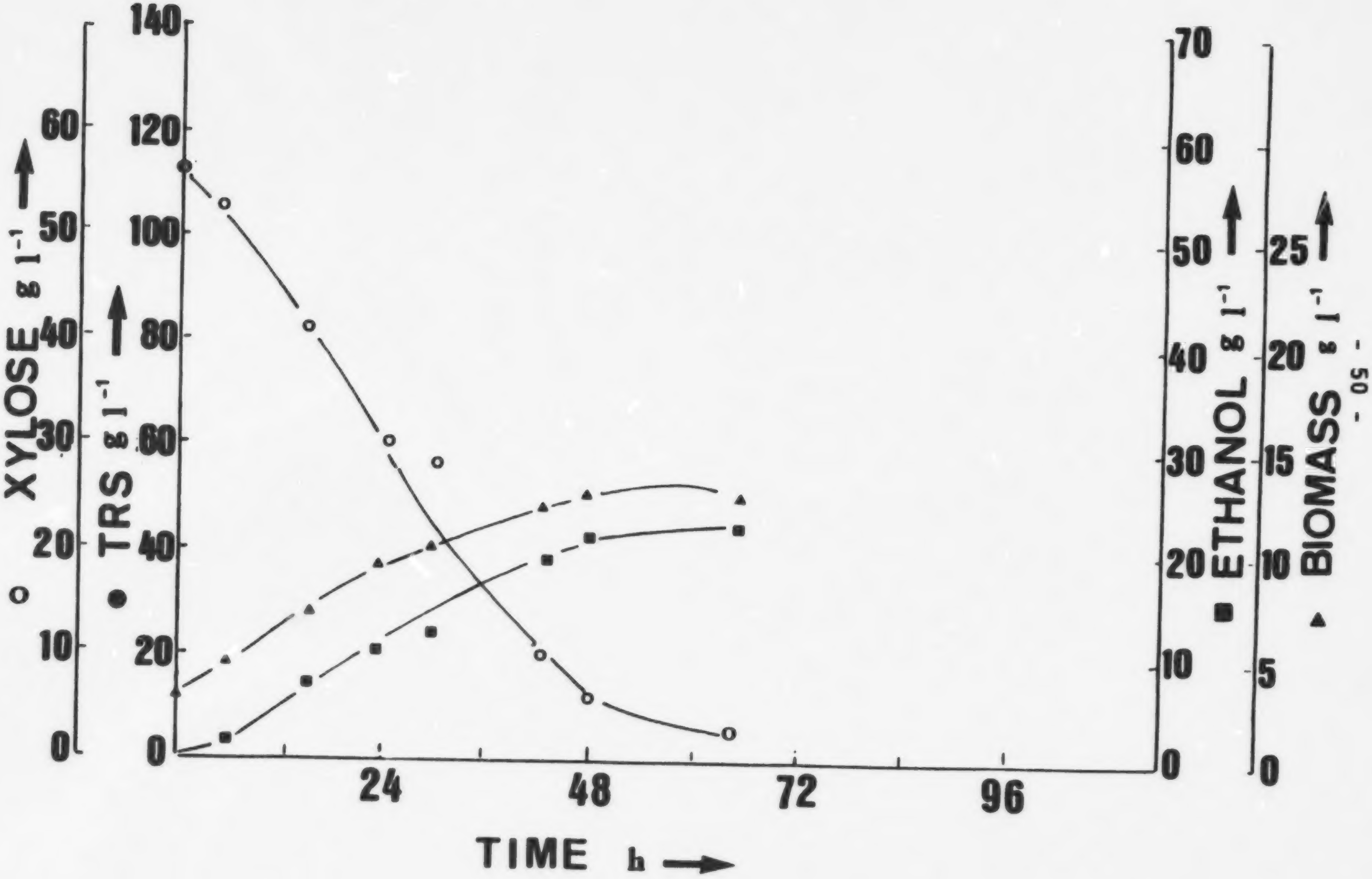

○ XYLOSE g l⁻¹ →
0
10
20
30
40
50
60
● TRS g l⁻¹ →
0
20
40
60
80
100
120
140
■ ETHANOL g l⁻¹ →
0
10
20
30
40
50
60
70
▲ BIOMASS g l⁻¹ →
0
5
10
15
20
25
24
48
72
96
TIME h →

Table No. 3
Experiment No. 3 Date July 17, 1988
Substrate glucose/xylose Medium, and medium preparation
g/x = 70/30; 20 L in 28 L fermenter; YMP @ 2, 2, 3 g/L; pH 5.0,
30°C, agitator 200 rpm, 19 ppm polypropylene glycol
yeast *Pichia stipitis* R

	t = 0	t = 24	t = 48	t = f (92h)
TRS g/L	130	50	24	7
fermentable sugars, g/L				
biomass g/L	0.38	7	12	12
ethanol g/L		38	49	55
aeration vvm	0.15	0.10	0.075	

fermentation time t = f 92 h
biomass OD, t = 0, 0.043 ; t = f, 0.42; at t = 0, 1/10; t = f, 1/100
" log OD, t = 0, .84 ; t = f .38
residual glucose (t = f) g/L 0
" xylose (t = f) g/L 7
" mannose (t = f) g/L 0

Yx/s	g biomass/g sugar consumed	0.098
Yp/s	g ethanol/g sugar consumed	.445
Vp	g ethanol/L-h	.59
Qp	g ethanol/g biomass(f)-h	.050

GRAPH * 3

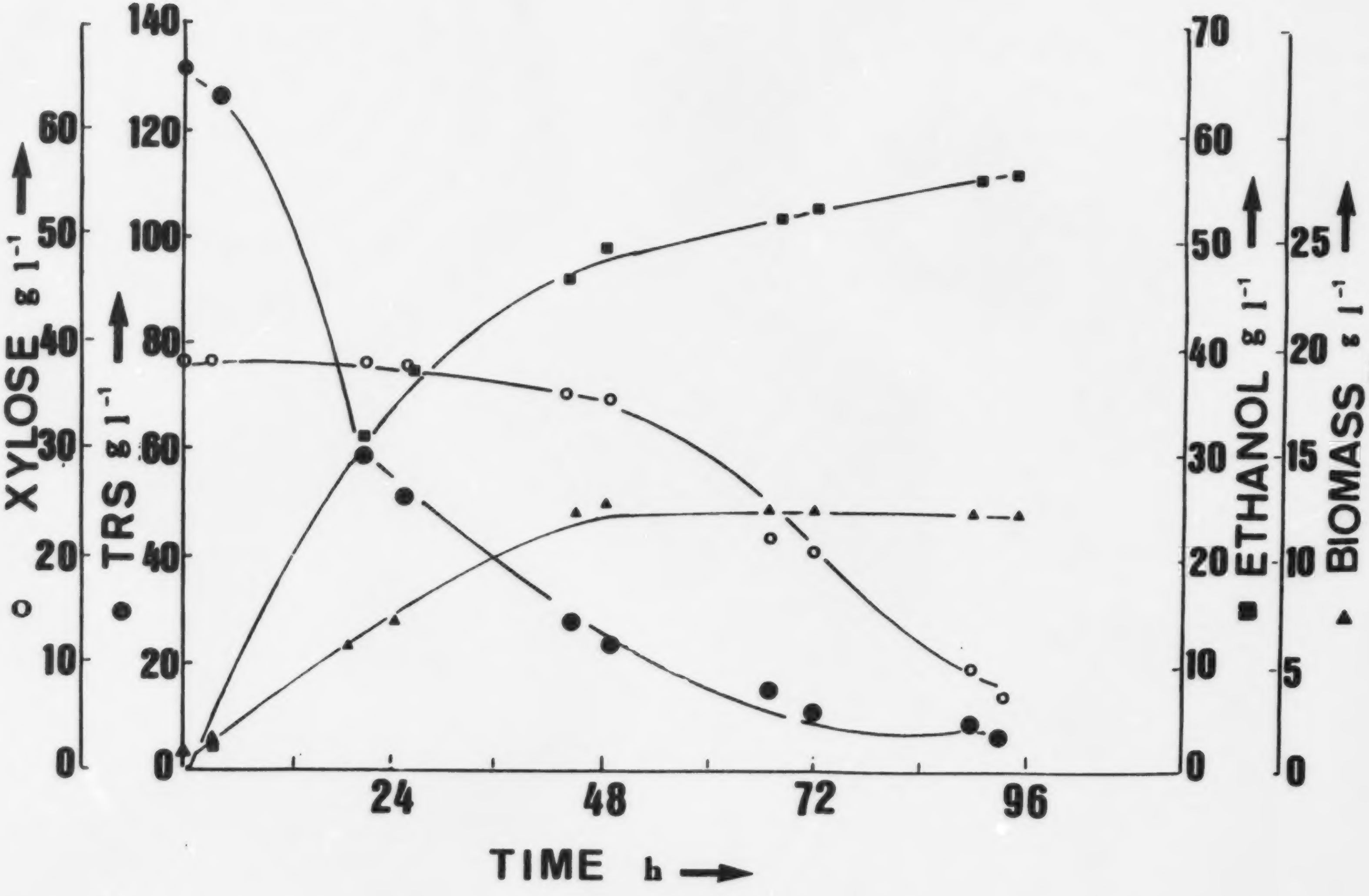

○ XYLOSE g l⁻¹ ↑
● TRS g l⁻¹ ↑
■ ETHANOL g l⁻¹ ↑
▲ BIOMASS g l⁻¹ ↑
0
10
20
30
40
50
60
0
20
40
60
80
100
120
140
0
10
20
30
40
50
60
70
0
5
10
15
20
25
24
48
72
96
TIME h →

Table No. 4
Experiment No. 4 Date June 30, 1988
Substrate aspen Medium, and medium preparation , steam stripped prehydrolysate; 15 L in 28 L fermenter; YMP @ 3, 3, 5 g/L
pH 5.9, 30°C,, agitator 200 rpm,15 ppm polypropylene glycol
yeast Pichia stipitis R

	t = 0	t = 24	t = 48	t = f (68 h)
TRS g/L	60			
fermentable sugars, g/L	58	29	18	6
biomass g/L	9	12	12	12
ethanol g/L		13	18	24

aeration vvm 0.20

fermentation time t = f 68 h
biomass OD, t = 0, 0.090 t = f, 0.137, 1/100
" log OD, t = 0 ; t = f
residual glucose (t = f) g/L 0
" xylose (t = f) g/L 4
" mannose (t = f) g/L 2 (others, arabinose)

Yx/s	g biomass/g sugar consumed	0.06
Yp/s	g ethanol/g sugar consumed	.44
Vp	g ethanol/L-h	.35
Qp	g ethanol/g biomass(f)-h	.10

GRAPH # 4

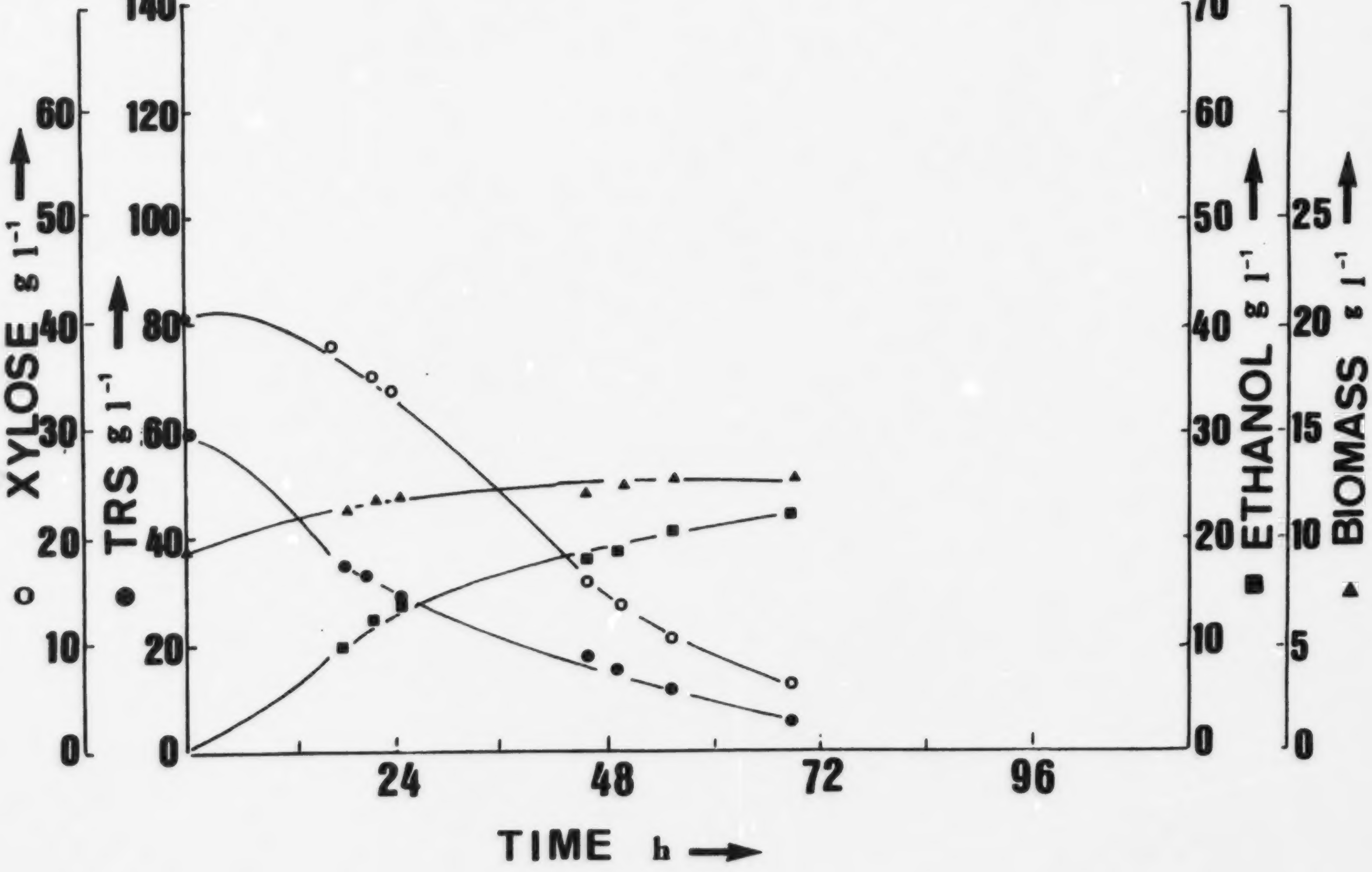

XYLOSE g l⁻¹ ○
60
50
40
30
20
10
0
TRS g l⁻¹ ●
140
120
100
80
60
40
20
0
ETHANOL g l⁻¹ ■
70
60
50
40
30
20
10
0
BIOMASS g l⁻¹ ▲
25
20
15
10
5
0
24
48
72
96
TIME h

Table No. 5
Experiment No. 5 Date August 22, 1988
Substrate aspen Medium, and medium preparation
hydrolysate; 2.7 L in 4 L fermenter; YMP @ 1, 1, 1 g/L
pH 5.5, 30°C, agitatot 200 rpm, 10 ppm polypropylene glycol
yeast Saccharomyces cerevisiae

	t = 0	t = 24	t = 48	t = f (68h)
TRS g/L	103			
fermentable sugars, g/L	90	83	23	12
biomass g/L	14	15	17	18
ethanol g/L		8	36	43

aeration vvm none

fermentation time t = f 68h
biomass OD, t = 0, 0.151 t = f, 0.194
" log OD, t = 0 .82; t = f .712
residual glucose (t = f) g/L 0
" xylose (t = f) g/L 12
" mannose (t = f) g/L

Yx/s	g biomass/g sugar consumed	0.050
Yp/s	g ethanol/g sugar consumed	.477
Vp	g ethanol/L-h	.63
Qp	g ethanol/g biomass(f)-h	.15

GRAPH # 5

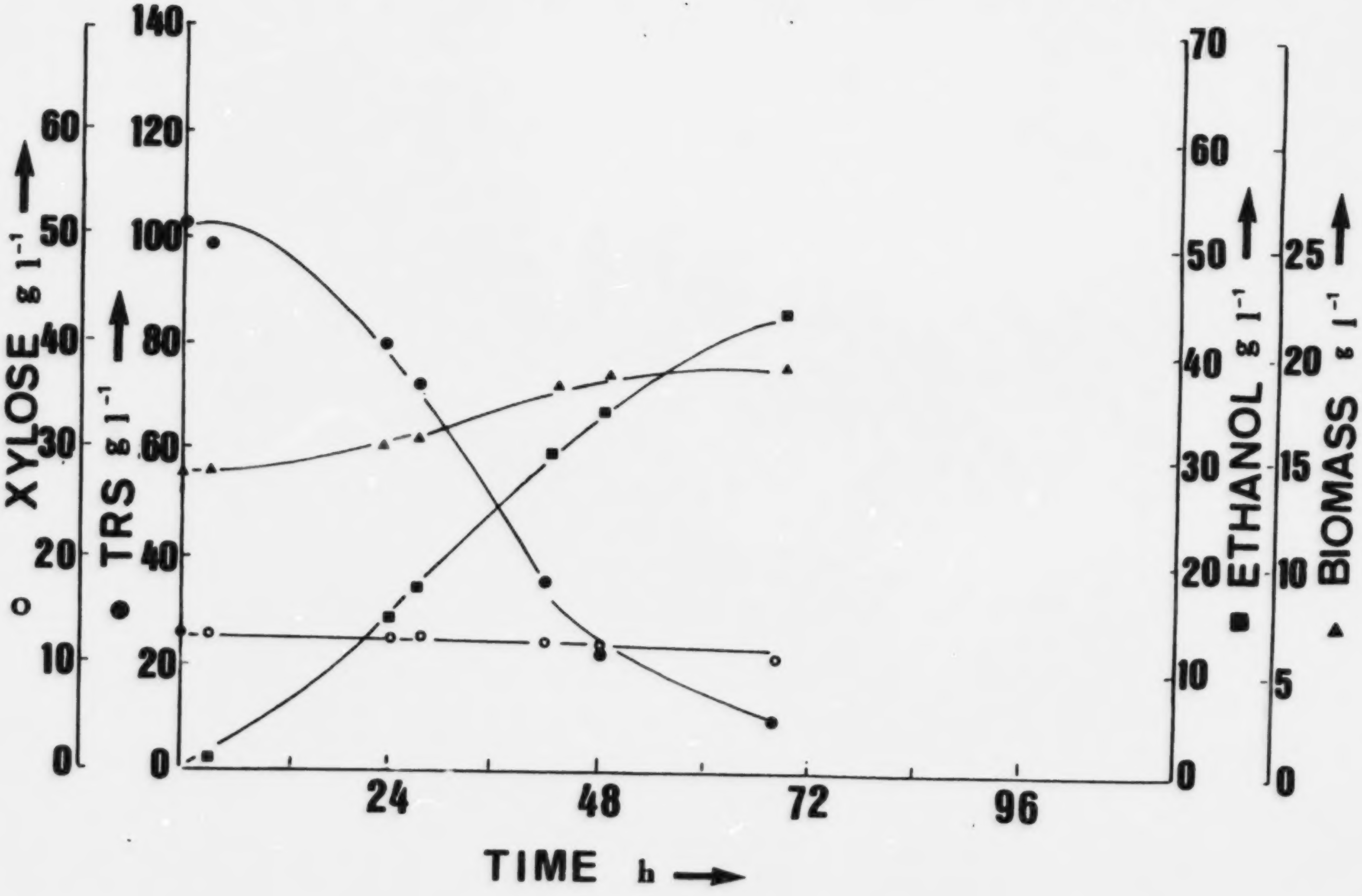

○ XYLOSE g l⁻¹
● TRS g l⁻¹
■ ETHANOL g l⁻¹
▲ BIOMASS g l⁻¹
TIME h
0
10
20
30
40
50
60
0
20
40
60
80
100
120
140
24
48
72
96
0
10
20
30
40
50
60
70
0
5
10
15
20
25

Table No. 6
Experiment No. 6 Date November 30, 1988
Substrate aspen Medium, and medium preparation
mixed prehydrolysate and hydrolysate, prehydrolysate steam stripped
15 L in 28 L fermenter, YMP 1,1,2 g/L, pH 5.2, 30°C, agitator 200 rpm
15 ppm polypropylene glycol, yeast *Pichia stipitis* R

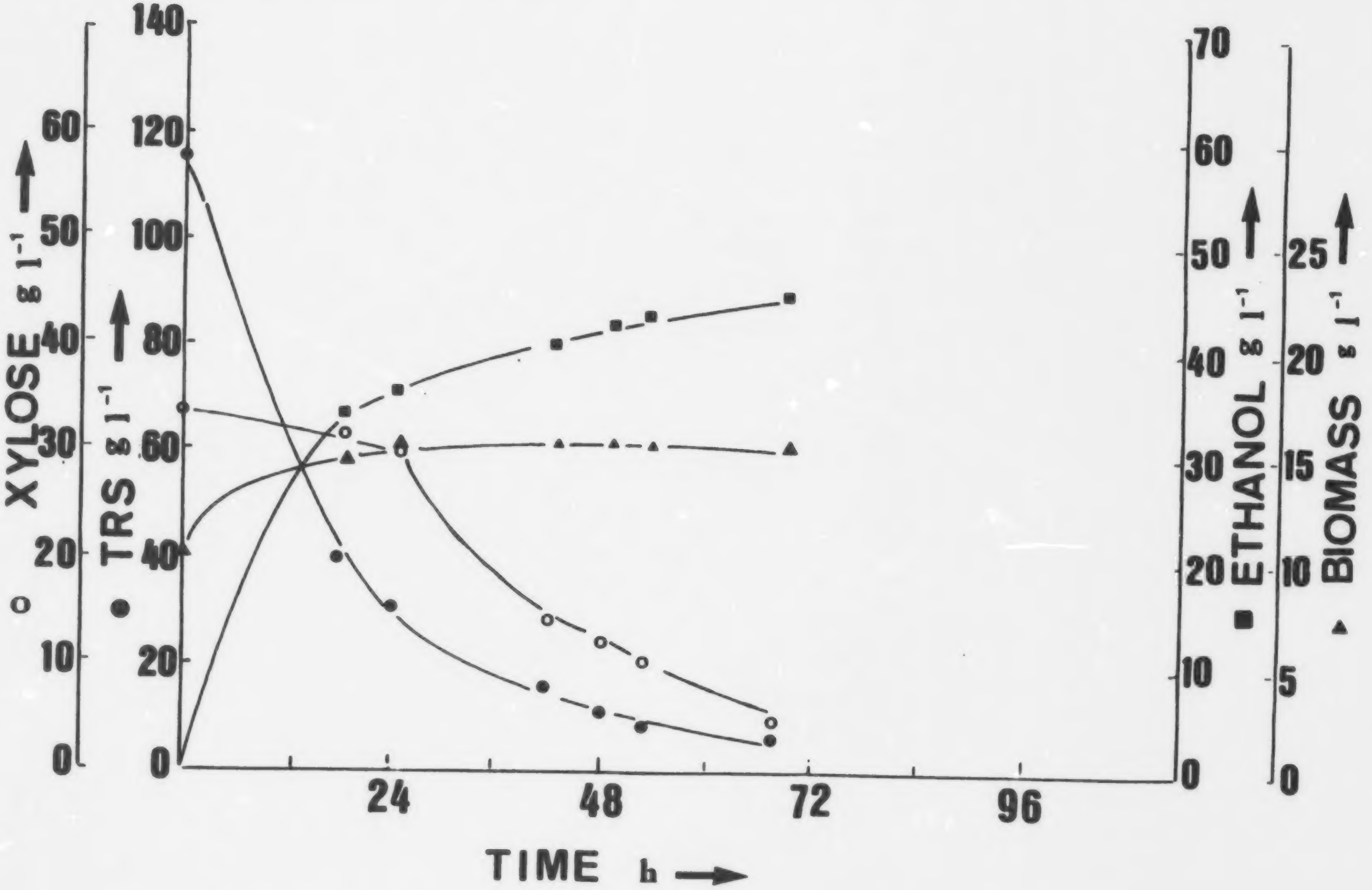
	t = 0	t = 24	t = 48	t = f (66h)
TRS g/L	116			
fermentable sugars, g/L	112	30	15	4
biomass g/L	10	15	15	15
ethanol g/L				46.5
aeration vvm	0.15	0.24	0.12	

fermentation time t = f 66 h
biomass OD, t = 0,0.11 ; t = f, 0.164; @ 1/100
" log OD, t = 0,0.95 ; t = f, .78
residual glucose (t = f) g/L 0
" xylose (t = f) g/L 4
" mannose (t = f) g/L 4 (others, arabinose)

Yx/s	g biomass/g sugar consumed	0.13
Yp/s	g ethanol/g sugar consumed	.43
Vp	g ethanol/L-h	.70
Qp	g ethanol/g biomass(f)-h	.14

0.42

GRAPH # 6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60
50
40
30
20
10
0
● TRS g l⁻¹ ↑
140
120
100
80
60
40
20
0
24
48
72
96
TIME h →
70
60
50
40
30
20
10
0
■ ETHANOL g l⁻¹ ↑
25
20
15
10
5
0
▲ BIOMASS g l⁻¹ ↑

Table No. 7
Experiment No. 7 Date July 9, 1988
Substrate pine Medium, and medium preparation
prehydrolysate, steam stripped; YMP 1,1,2 g/L;
2.7 L in 4 L fermenter; pH 5.3, 30°C, agitator 200 rpm
10 ppm oolyethylene glycol; yeast *Pichia stipitis* R

	t = 0	t = 24	t = 48	t = f (41h)
TRS g/L	53			
fermentable sugars, g/L	52	11		5
biomass g/L	16.7	22		22
ethanol g/L		21		23.5

aeration vvm 0.15

fermentation time t = f 41 h
biomass OD, t = 0, 0.18 ; t = f 0.24; @ 1/100
" log OD, t = 0 .74 ; t = f .61
residual glucose (t = f) g/L 0
" xylose (t = f) g/L 4
" mannose (t = f) g/L 1 other (arabinose)

Yx/s	g biomass/g sugar consumed	0.11
Yp/s	g ethanol/g sugar consumed	.49
Vp	g ethanol/L-h	.56
Qp	g ethanol/g biomass(f)-h	.10

GRAPH * 7

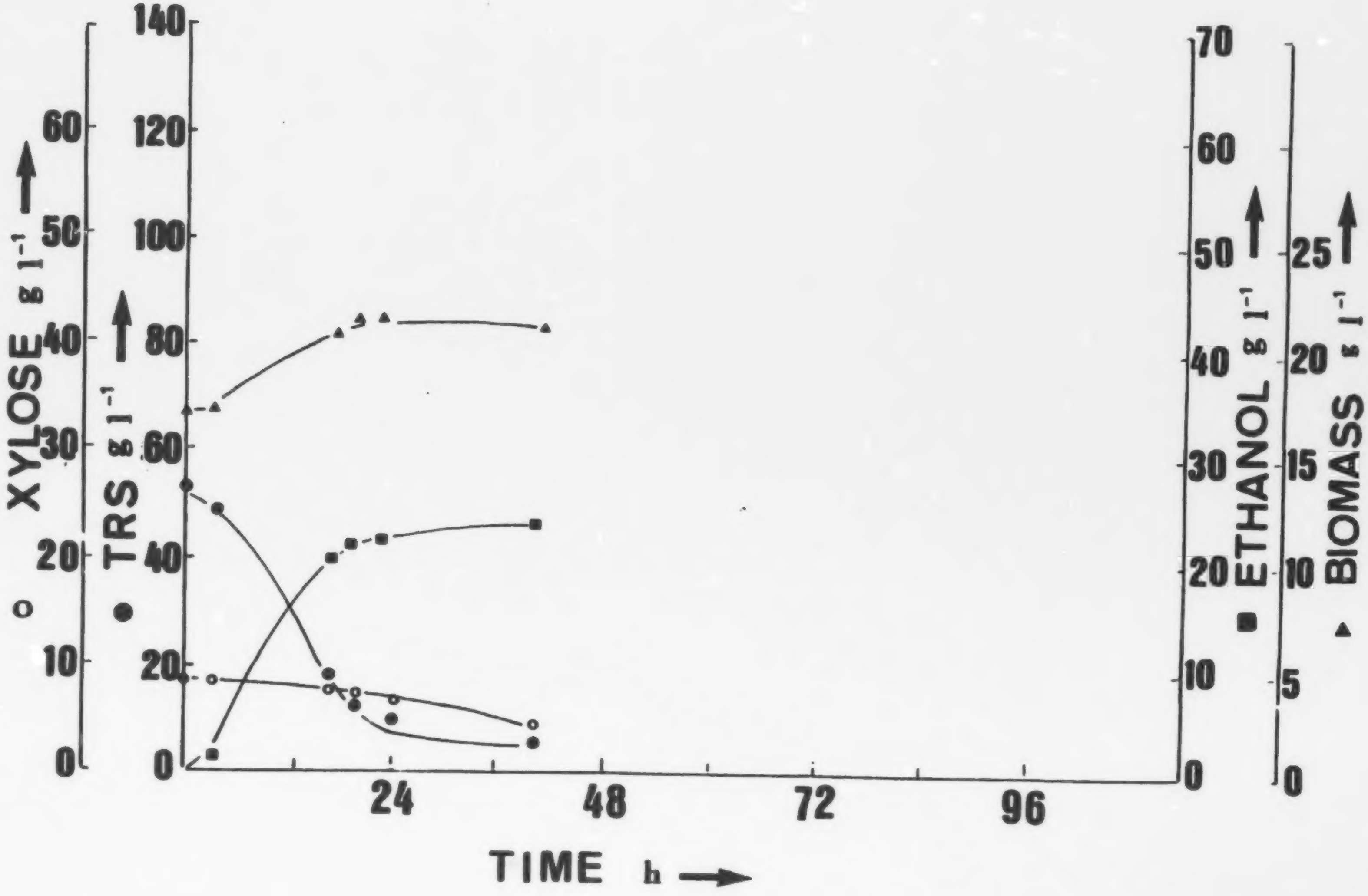

○ XYLOSE g l⁻¹
0
10
20
30
40
50
60
● TRS g l⁻¹
0
20
40
60
80
100
120
140
■ ETHANOL g l⁻¹
0
10
20
30
40
50
60
70
▲ BIOMASS g l⁻¹
0
5
10
15
20
25
TIME h
24
48
72
96

Table No. 8
Experiment No. 8 Date July 15, 1988
Substrate pine Medium, and medium preparation
hydrolysate; 2.7 L in 4 L fermenter; YMP 1,1,1 g/L;
pH 5.3, 30°C, agitator 200 rpm, 10 ppm peg antifoam
yeast *Pichia stipitis* R

	t = 0	t = 24	t = 48	t = f (12 h)
TRS g/L	78.5	42 (8 h)	1.5 (12 h)	
fermentable sugars, g/L				
biomass g/L	14	17	18	
ethanol g/L		18	35	

aeration vvm 0.1

fermentation time t = f 12 h
biomass OD, t = 0, 0.155; t = f, 0.193
" log OD, t = 0 .80 ; t = f .714
residual glucose (t = f) g/L 0
" xylose (t = f) g/L 1.5
" mannose (t = f) g/L 0

Yx/s	g biomass/g sugar consumed	0.04
Yp/s	g ethanol/g sugar consumed	.45
Vp	g ethanol/L-h	2.9
Qp	g ethanol/g biomass(f)-h	.85

GRAPH # 8

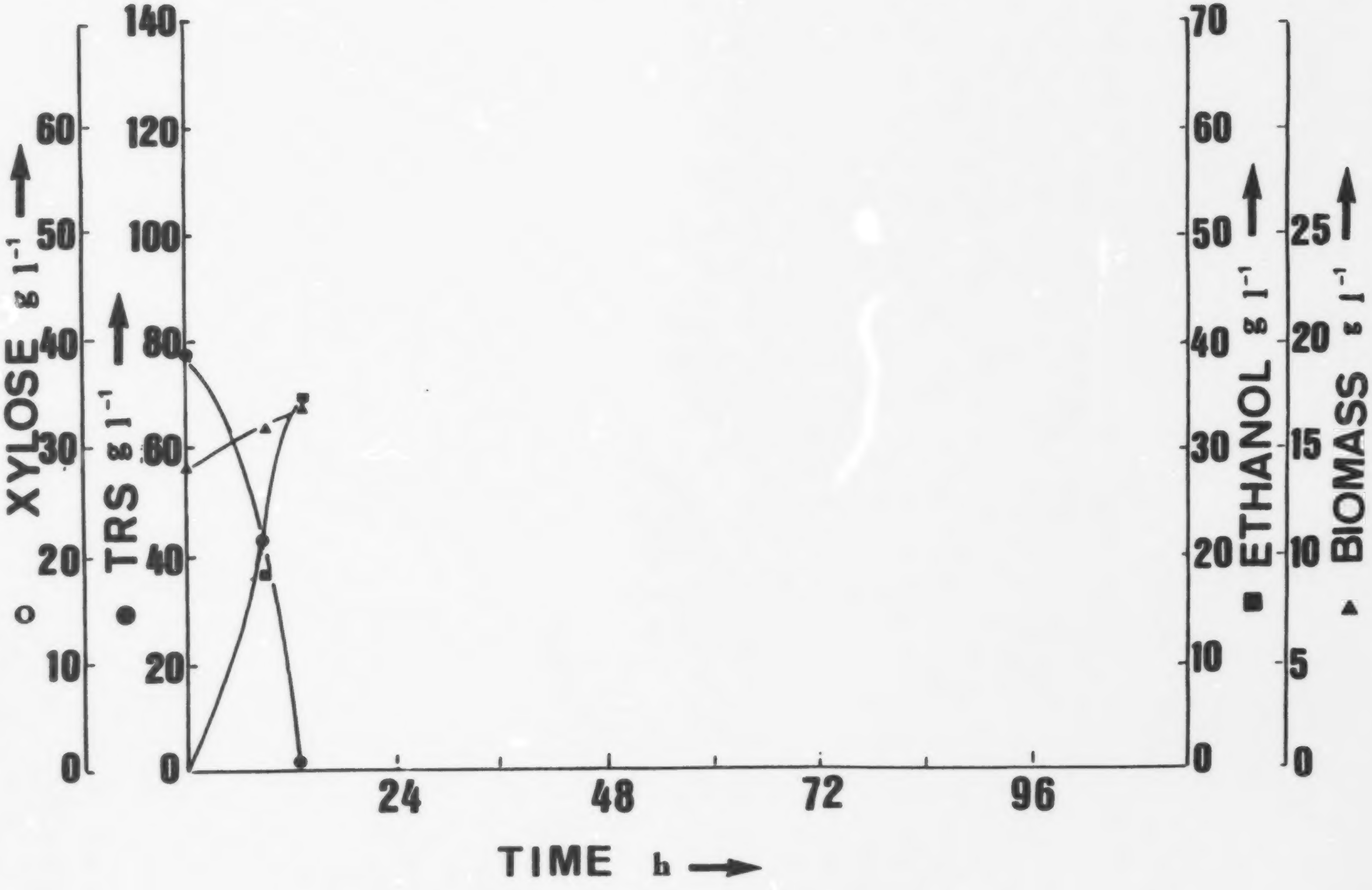

○ XYLOSE g l⁻¹
0
10
20
30
40
50
60
● TRS g l⁻¹
0
20
40
60
80
100
120
140
■ ETHANOL g l⁻¹
0
10
20
30
40
50
60
70
▲ BIOMASS g l⁻¹
0
5
10
15
20
25
24
48
72
96
TIME h

Table No. 9
Experiment No. 9 Date August 7, 1988
Substrate pine Medium, and medium preparation
steam stripped prehydrolysate + hydrolysate; 2.5 L in 4 L fermenter
YMP 1,1,2 g/L,pH 5.4, 30°C, agitator 200 rpm, 20 ppm peg atifoam,
yeast Pichia stipitis R

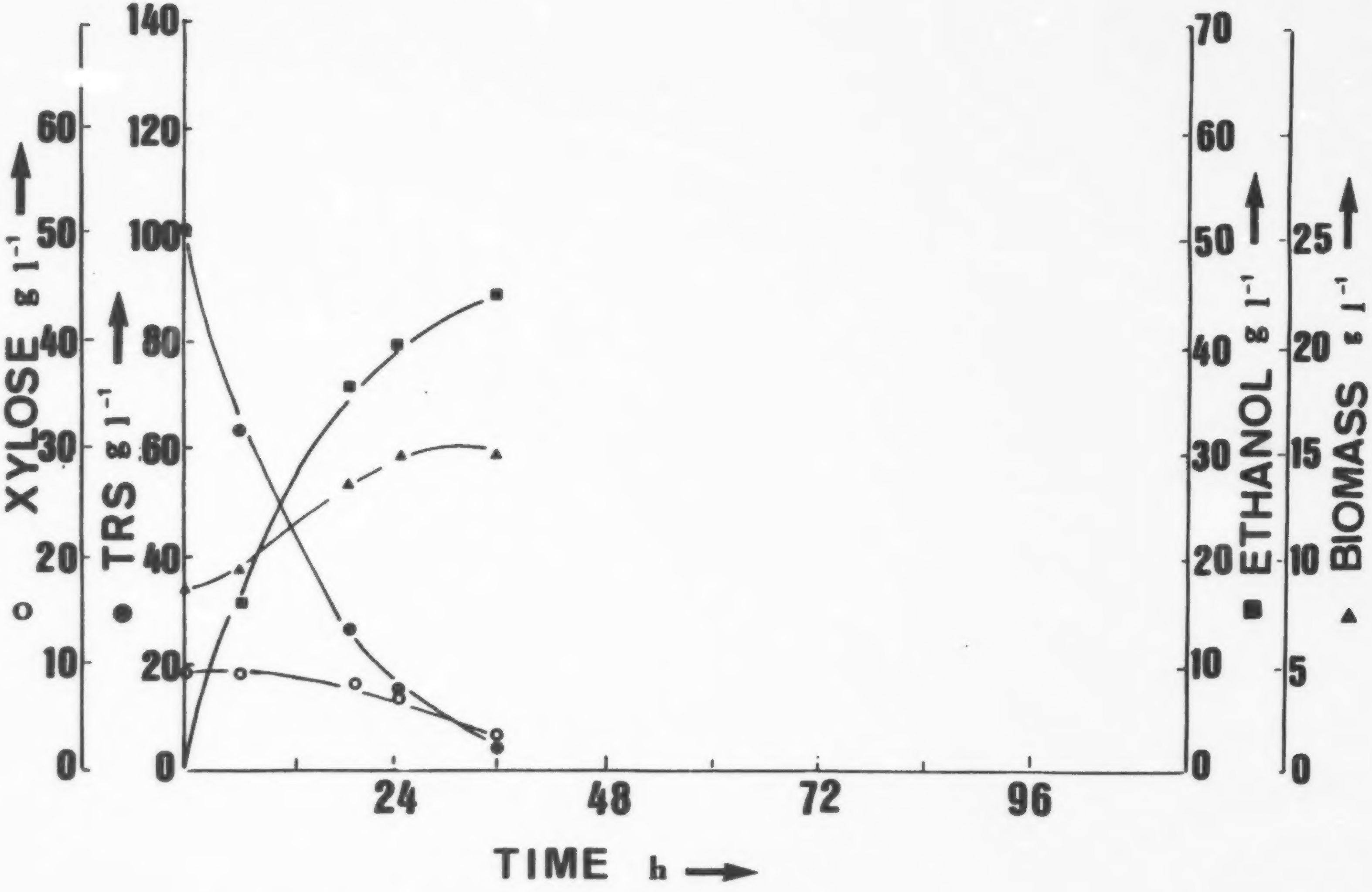
	t = 0	t = 24	t = 48	t = f (36h)
TRS g/L	102			
fermentable sugars, g/L	99	18		5
biomass g/L	8	14		14
ethanol g/L		40		45.3

aeration vvm 0.25 0.15

fermentation time t = f 36 h
biomass OD, t = 0,0.092 ; t = f 0.155; @ 1/100
" log OD, t = 0 1.03 ; t = f .809
residual glucose (t = f) g/L 0
" xylose (t = f) g/L 2
" mannose (t = f) g/L 3 (other, arabinose)

Yx/s	g biomass/g sugar consumed	0.060
Yp/s	g ethanol/g sugar consumed	.45
Vp	g ethanol/L-h	1.29
Qp	g ethanol/g biomass(f)-h	.21

GRAPH # 9


○ XYLOSE g l⁻¹
0
10
20
30
40
50
60
● TRS g l⁻¹
0
20
40
60
80
100
120
140
■ ETHANOL g l⁻¹
0
10
20
30
40
50
60
70
▲ BIOMASS g l⁻¹
0
5
10
15
20
25
24
48
72
96
TIME h

Discussion of Tables and Graphs.

1. Ethanol produced per gram sugar consumed was satisfactory in all cases, varying between 0.43 and 0.49, averaging 0.456 g/g. The efficiency of conversion of xylose was also high.

2. Fermentation time covered a very broad range, from 12 h to 92 h. For xylose pure sugar in 2 examples, fermentation time was 32 and 64 h, and the longest fermentation time, 92 h, was for the glucose-xylose pure sugar mixture. This latter fermentation began with only 0.38 g/L yeast cells, which meant a long lag period while cell concentration built up.

   The 3 aspen fermentations took about 68 h, while the 3 pine fermentations took 41, 12 and 36 h respectively, suggesting that the pine hydrolysis products are less inhibited than the aspen hydrolysis products. The aspen hydrolysate was fermented with common bakers' yeast (Table 5, p 55 and 56), in about the same time as the R yeast fermentation. The shortest fermentation, 12 h, was pine acid hydrolysate (Table 8, p 61 and 62) using the R yeast. It contained 77 g/L glucose + 1.5 g/L xylose. The 4.4 % by volume alcohol solution so produced is more dilute than we would aim for in industrial fermentation; we have no doubt that a more concentrated pine wood glucose solution, say 150 g/L, would produce 8.5 % by volume ethanol, a more desirable concentration, in less than 24 h.

3. Initial biomass was highly variable, from 0.38 to 16.7 g/L. This was due to the difficulty of producing large inocula for the 28 L fermenter with our laboratory equipment. Eventually a laboratory procedure was developed which enabled us to harvest sufficient yeast cells for inoculation of the larger fermenter at reasonable cell concentrations, 8 - 16 g/L. We would have preferred 20+ g/L. The fermentation with the lowest initial cell concentration (Table 3) was the slowest.

4. There was considerable difficulty in operating the 28 L fermenter for quite a long time, until that got straightened out. The difficulties were mainly around temperature and agitator speed control, both quite important.

5. We wanted to control dissolved oxygen, but the necessary meters were not available. For this reason it is difficult to draw firm conclusions from the aeration rates reported here. The first xylose fermentation, (Table 1), in the 4 L fermenter, was good, reasonably rapid, high efficiency of xylose conversion. The second xylose fermentation, (Table 2), and the xylose-glucose fermentation (Table 3), both in the 28 L fermenter, followed the same aeration pattern, but not the specific rates of the first, which may account in part for their longer fermentation times.

6. In these runs, the nutrient used was YMP (yeast extract, malt extract, peptone), a standard used by most workers in this field, which we used at quite low levels, 1 g/L each, although in the earlier fermentations more YMP was used. This decision was taken because of our inability in the earlier runs to provide enough inoculum. We have always used YMP in the laboratory to prepare inoculum, and we consider that these runs were in a sense a combination of yeast growth and fermentation.

   In our shake flask fermentations we use YMP to prepare inoculum, and DAP-U (diammonium phosphate-urea) as nutrient in fermentation. We did not use DAP-U in these runs. We were having quite a bit of mechanical difficulty in these early runs, and we wanted the yeast to have the best chance possible. Once YMP was adopted as standard for this set of runs, we were loathe to change it, because it would have made interpretation difficult.

   It is most unfortunate that we did not turn our attention to corn steep liquor as fermentation nutrient until after our laboratory work on this contract was terminated. We would now recommend that, following proper testing, corn steep liquor be adopted as fermentation nutrient. This is discussed in Section 17, page 26, of this Report.

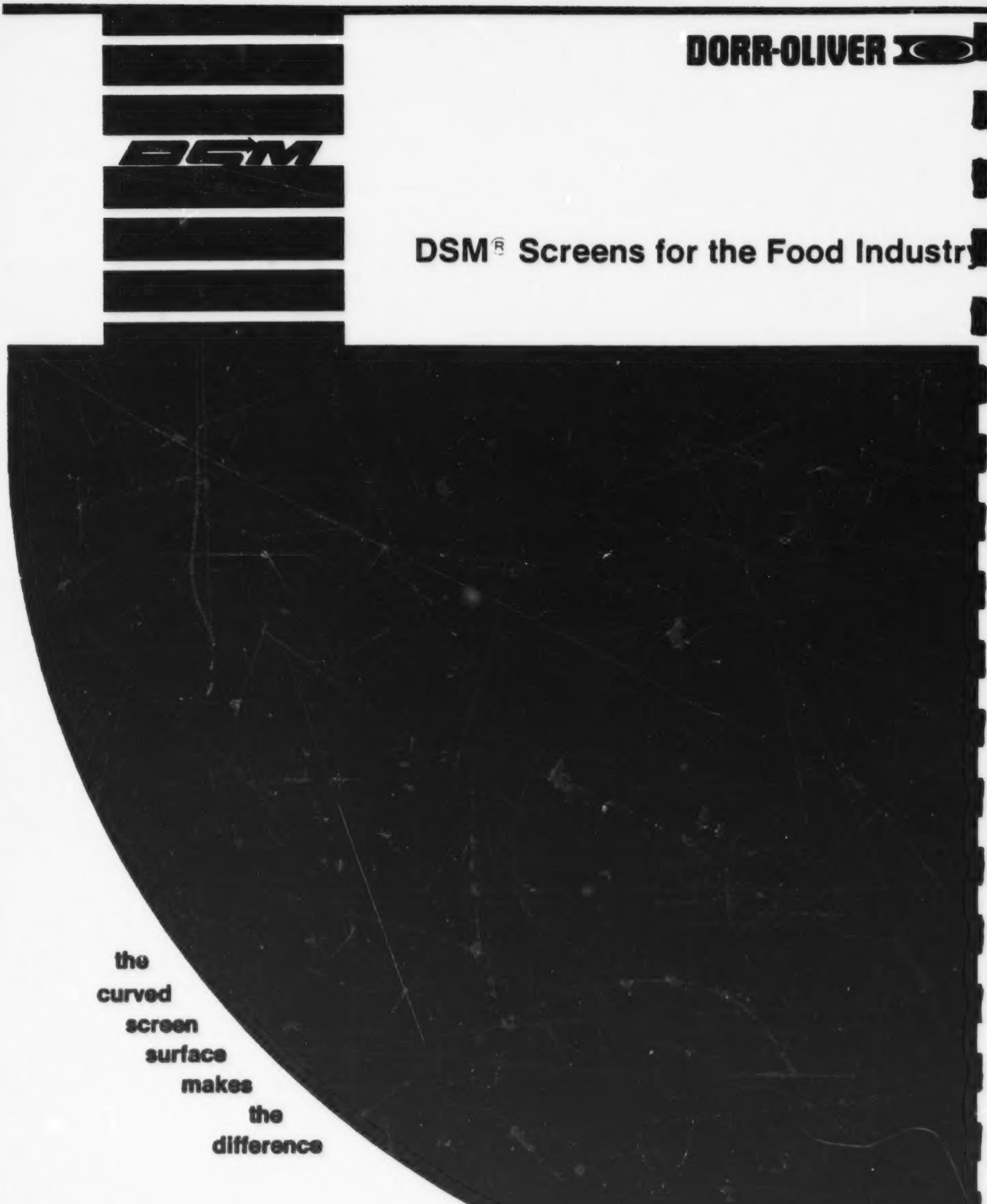
7. To sum up, aspen and pine hydrolysates are easy to ferment; steam stripped aspen and pine prehydrolysates ferment readily; mixtures of aspen or pine hydrolysates with their steam stripped prehydrolysates ferment readily. Aspen hydrolysis products ferment more slowly than pine hydrolysis products, but with the same good conversion efficiency. Prolonged runs with cell recycle would result in more rapid, and perhaps even more efficient, fermentation.

APPENDIX VI

DORR-OLIVER DSM SCREENS

SPECIFICATIONS

DORR-OLIVER



DSM

DSM® Screens for the Food Industry

the curved screen surface makes the difference

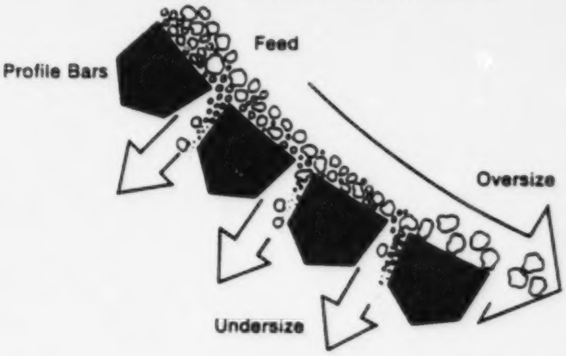
here's how the DSM works

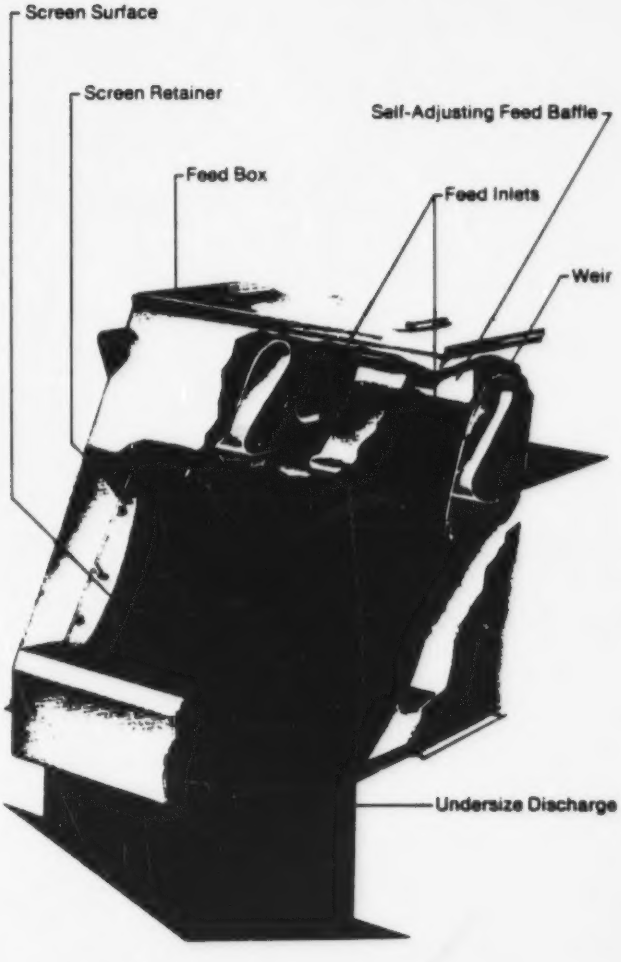
The DSM® is a rugged stationary screening device employing a concave wedge bar type of screen used in a variety of high capacity wet screening applications covering a wide range of separations. In operation, the feed to the screen is introduced tangentially at the proper velocity and flows across the concave screen surface at right angles to the openings between the wedge bars. Each of the wedge bars acts as a knife on the underside of the passing slurry. A layer of liquid and undersize solid particles strikes the sharp leading edge of the bar and is sliced off and directed downward through the slot into the screen box. The layer peeled off by this unique slicing action produces undersize particle separations smaller than the size of the wedge bar openings, thus preventing blinding of the screen. The oversize material continues to flow across the screen being continually dewatered as it moves along and is discharged at the far end of the screen. The volume of undersize that passes through the openings between the wedge bars is largely determined by how well the slurry is held in contact with the screen surface. The continuously curved surface design which is unique to the Dorr-Oliver DSM Screen and the velocity across the surface provide this holding action by the centrifugal force created. The DSM, therefore, produces high-capacity, efficient separation without blinding and a well-dewatered, coarse oversized material.

The size separation made by the DSM Screen is dependent upon the spacing between the wedge bars and the velocity of the material flowing across the curved surface. The finer the mesh of separation, the higher the velocity required. For this reason, two basic screen designs are utilized by Dorr-Oliver. 1. The screens employed for separations down to approximately 65 mesh are gravity fed screens employing a parabolic feed weir which provides an evenly distributed flow introduced tangentially onto the screen surface and because of the height of the weir, a sufficient velocity is produced for the centrifugal force required for good dewatering rates. 2. When finer separations are required, the feed slurry is tangentially introduced to the screen surface through nozzles at velocities sufficiently high to insure that the screen functions efficiently.

the
curve
makes
the
difference

The continuously curved surface design, unique to the Dorr-Oliver DSM Screen, together with the velocity across the surface provides a centrifugal force which holds the slurry against the screen surface constantly.





and this is how well it works

things you need to know about installing the DSM

Feed Conditions

Gravity Fed Screens

The 45° and 50° DSM Screens, which employ a parabolic feed weir, require no special feed conditions other than sufficient head to flow feed into the feed box for the screen. The feed inlet to the screen is at the bottom of the feed compartment which provides a rising, evenly distributed flow over the weir and prevents settling of gross oversize material in the feed compartment.

Pressure Fed Screen

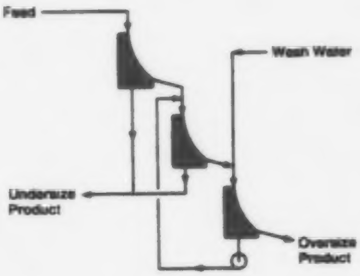
The 120° and 300° DSM Screens employ nozzles feeding tangentially to the screen and require pressures in the order of 30-60 lbs./sq. in. depending upon the mesh of separation of the screen used. When these units are utilized, the pump conditions should be selected based on the feed pressure requirement and the total flow that will be handled by the screen. These pressure and flow conditions are shown on Page 8.

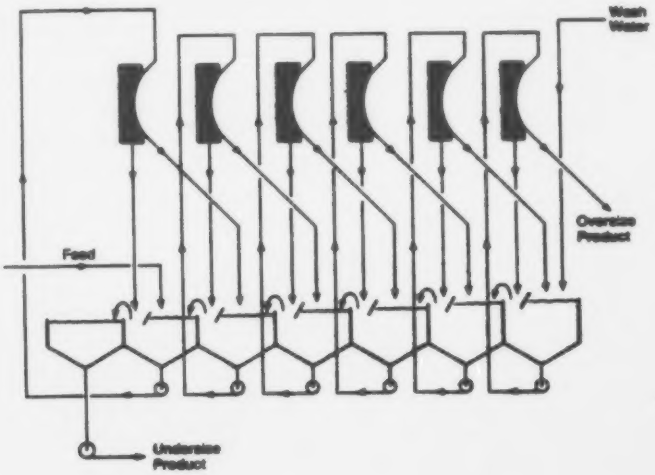
Maximum Size of Solids to be Handled by the DSM—The gravity fed screens, which employ a parabolic weir feeding tangentially to the screen surface, can accommodate gross oversize material since there are no narrow passageways which can become clogged. Any gross oversize material flowing over the weir onto the screen surface can be discharged with the oversize product. With the pressure fed screens, the maximum particle size which can be handled by the unit is determined by the size of the nozzle. These nozzle sizes are shown on Page 8.

Condition of Discharges—The undersize fraction from the screen contains essentially all the water and is discharged by gravity at the bottom or the back of the screen. There is no residual pressure in the discharging of the undersize material even with pressure fed screens. The oversize material slides off the edge of the screen in a dewatered condition but is usually wet enough so that some dripping can occur.

Method of Conveying Oversize and Undersize—The undersize material flows away from the screen by gravity or is pumped away. The oversize material can be discharged into holding bins or tanks or conveyed by screw conveyors and in some cases by belt conveyors. If it is desirable to remove more of the surface moisture from the oversize solids, perforated belt conveyors are sometimes used and the water from the oversize returned back to the feed of the screen.

Manifolding Series and Parallel—DSM Screens can be placed in series operation and often with a mixture of screens. For example, a gravity fed screen for coarse separation might be used ahead of a pressure fed screen making a very fine separation. For large installations requiring a single stage multi-screen system, the screens can be manifolded to be fed in parallel.





the DSM saves you money

High Capacity—The DSM handles extremely large flow volumes per unit of screening area because of the centrifugal force developed by use of a continuously curved surface coupled with the proper velocity of material across the screen. This high unit capacity means less equipment to do the job.

Non-clogging—Since the size of separation is determined by the thickness of the layer of material "sliced off," the DSM design eliminates blinding and therefore costly cleaning costs. The elimination of blinding also insures more complete recovery of desired mesh of separation and better recovery of valuable products.

Permanent Screen Media—The rugged wedge bar screen provides a permanent screening media eliminating costly screen replacements. The permanent screening media also eliminates possible contamination of undersize with oversize by screen breakage thus preventing loss of valuable products. The screen surface is reversible for increased life.

No Moving Parts—The DSM is a stationary, non-vibrating screening device which assures lower maintenance cost and insures long dependable service.

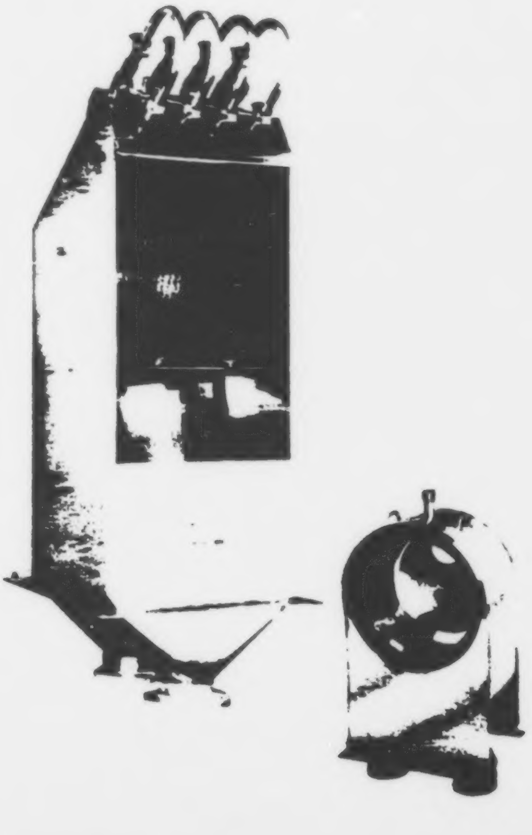
Low Space Requirement—The high capacity per unit area of the screen means lighter weight, smaller size units, and the simple design means both lower installation costs and lower initial capital cost.

the DSM is versatile

Sizing & Dewatering—The wide range of separations which can be made by the DSM, makes it possible to separate solids over a wide range of sizes. Often the same type of screen for all sizing applications can be utilized.

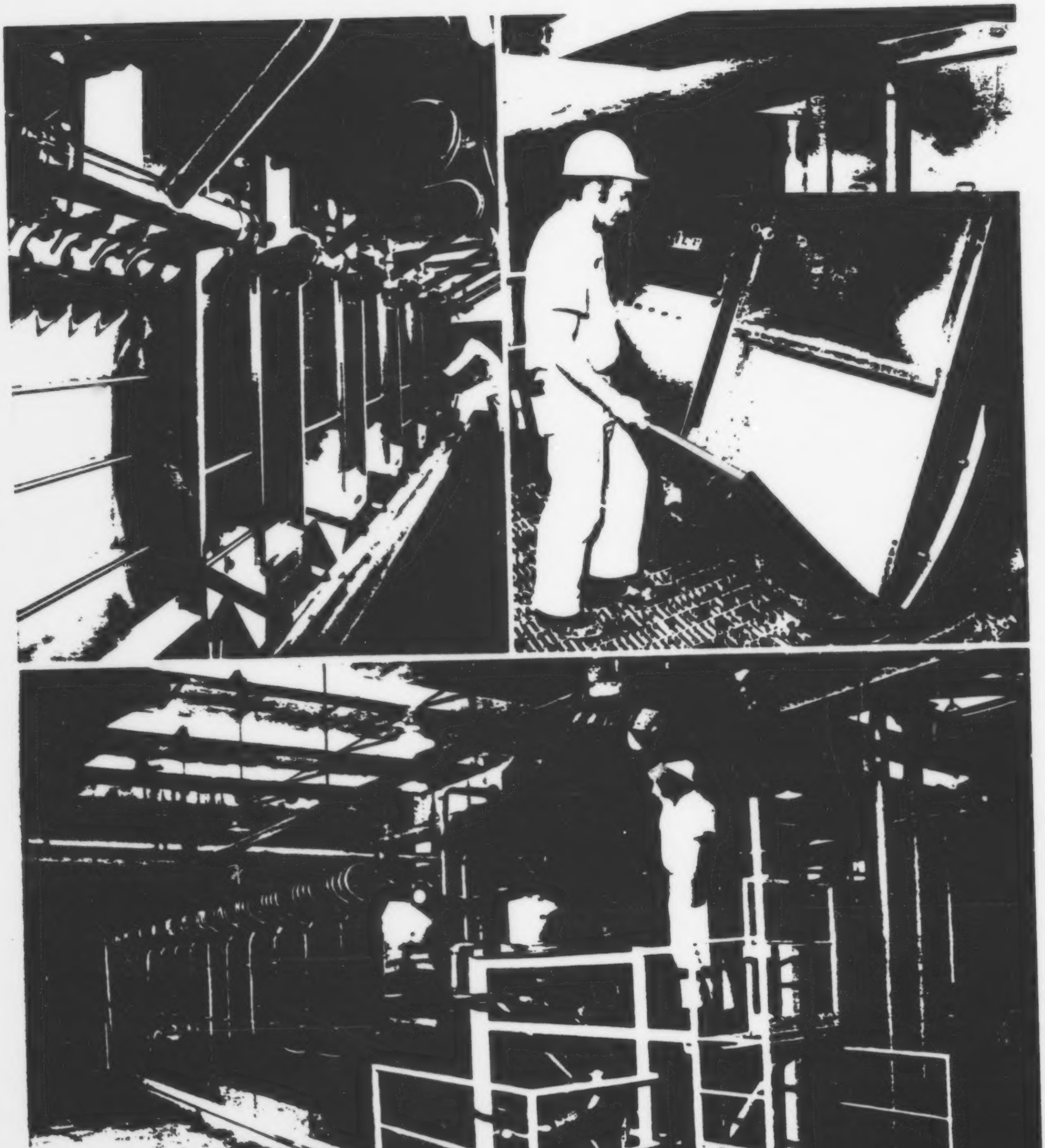
Washing and Extraction Including Multi-Stage—When it is necessary to screen out a material of a certain size range and wash contaminants from this oversize material, the DSM Screen can be used in multi-stage, counter-current systems or in single-stage systems for most efficient use of the wash water.

Waste Solids Removal—Many DSM Screens are used for removing solids from waste streams having particles with a wide range of particle size. By proper selection of screen or screens and proper staging, the solids can be removed down to any desired size level as small as 325 mesh.

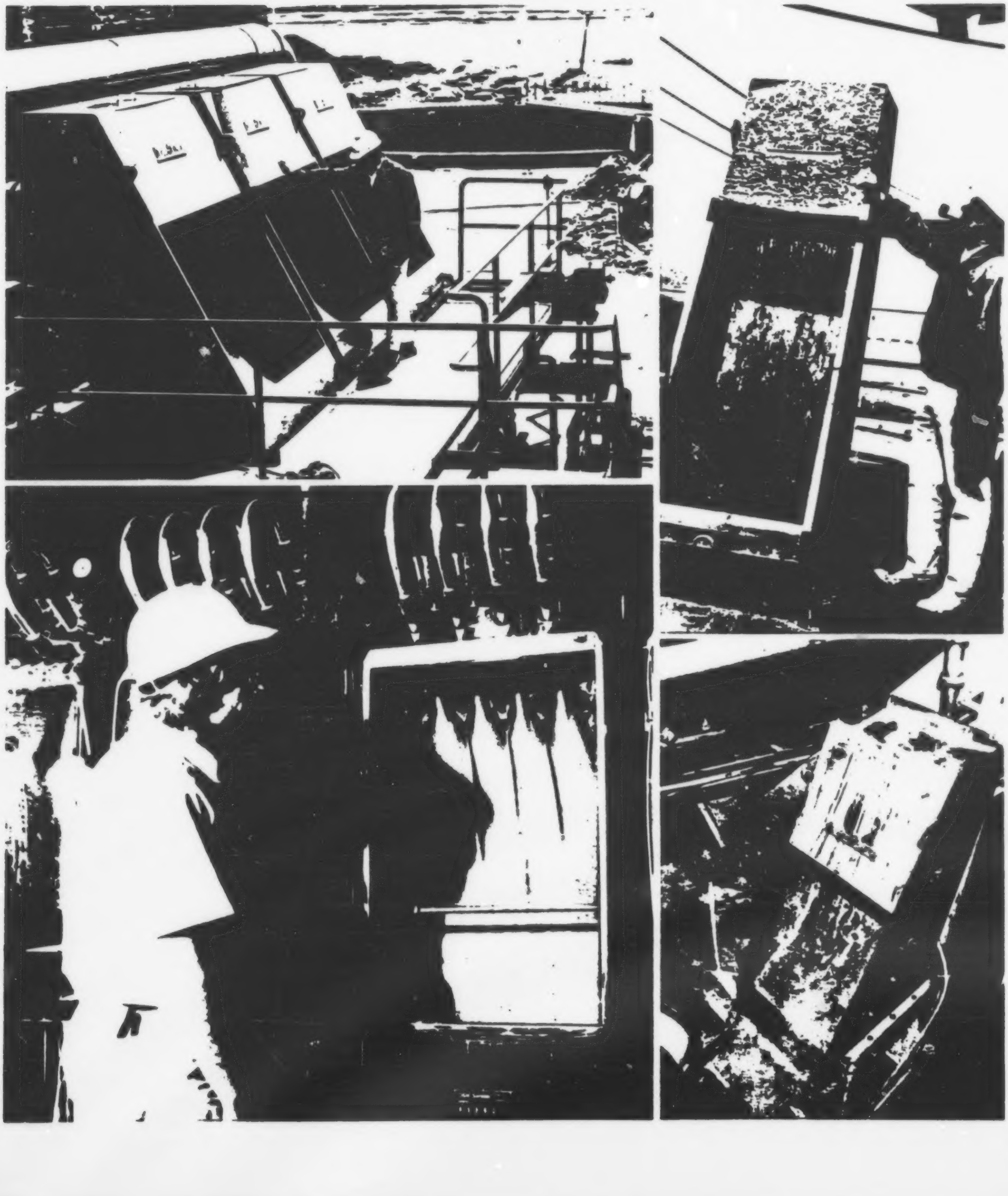


Important Advantage
(applies to 120° and 300° types)—
in fine screen applications, Dorr-Oliver has a
proprietary process for finishing the screen
surface that enables the DSM Screen to function
better than any other known surface.

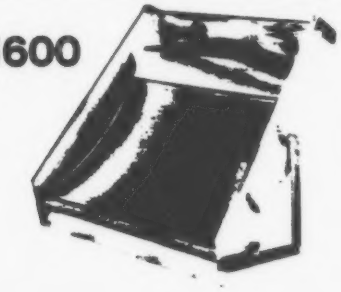
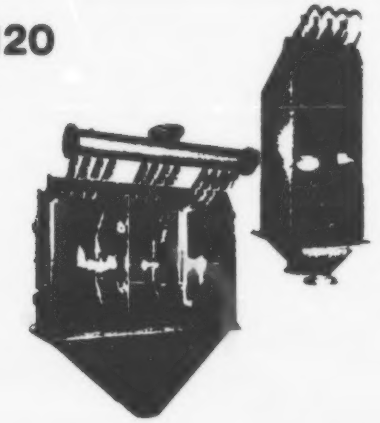
some typical installations of DSM screens in



food processing plants worldwide



There's a DSM to meet your requirements

MODEL	DESCRIPTION	APPLICATION
50-800	All stainless steel construction; weir fed; integral feed compartment; open bottom discharge; oversize discharge apron; rear inspection opening in undersize compartment; screen surface length 800 mm.	Dewatering or classification of easy to dewater solids. Corn, beans, peas, corn germ, beet pulp, hops, fruit and vegetable pulps.
45-1600 	All stainless steel construction; weir fed; integral feed compartment; open bottom discharge; oversize discharge apron; rear inspection opening in undersize compartment; screen surface length 1600 mm.	Dewatering or classification of difficult to dewater solids. Spinach, sugar cane, citrus waste, fish waste, vegetable waste.
120 	All stainless steel construction; pressure fed; over and underflow hopper bottoms, flanged; completely enclosed box; sanitary construction. Large front and rear doors. Screen surface length 1600 mm.	Fine fiber separation, corn, raw sugar solution, vegetable and citrus juice, biomass slurry.
300	All stainless steel construction; pressure fed; over and underflow hopper bottom with Victaulic connections; completely enclosed box; large front door, very easy to service; small size; sanitary construction; screen surface length 800 mm.	Same as 120 but lower flows; also wheat and potato starches.

technical data

MODEL		GRAVITY FED DSM SCREENS						PRESSURE FED DSM SCREENS			
		50-800			45-1600			120			300
WIDTH (nominal)	ft mm	2 600	4 1200	6 1800	2 600	4 1200	6 1800	2 600	4 1200	6 1800	8 inch 200
HEIGHT	in mm	67 1700			92 2340			88 2240	116 2950	116 2950	29 735
WEIGHT**	lbs kg	460 210	770 350	980 445	790 360	1080 490	1445 656	650 295	1000 450	1500 680	110 50
SCREEN SURFACE LENGTH	in mm	31.5 800			63 1600			63 1600			31.5 800
CAPACITY*	gpm/ft lpm/ft	30-150 115-570			30-200 115-760			SEE CHART BELOW			

*Depends on solids loading and slot width.

**Stainless steel construction. The gravity fed DSM screens are also available in mild steel construction.

		PRESSURE FED SCREEN CAPACITY			
Screen slot opening Microns		50	75	100	150
Approx. mesh of separation		325	250	170	120
Recommended Feed Pressure	psi atm	30-60 2-4			
Feed Nozzle Opening 120°	in²	.5	.75	1.0	1.0
Feed Nozzle Opening 300°	in²	.25	.5	.75	1.0
Flow Range* 120° (Per 2' width)	gpm lpm	150-200 570-760	180-300 680-1150	240-400 910-1500	300-500 1150-1890
Flow Range* 300° (Per 8" width)	gpm lpm	20-40 75-150	30-60 115-230	40-80 150-300	60-120 230-450

*Actual capacity depends on feed solids content, fiber size, shape of oversize solids and desired wetness of oversize stream.

SPECIAL POLISHING

In order to obtain superior performance with screen surfaces having slot widths of less than 200 microns, it is necessary to manufacture them with great precision and polish them extensively.

Process

A proprietary hydro grit finishing process was developed by Dorr-Oliver in conjunction with the Dutch State Mines. This process requires long hours of patient treatment to carefully shape every bar edge.

What does it do

Hydro grit finishing removes surface imperfections on both the top and on the side surfaces of the profile bars. It polishes and specially shapes the "leading" and "trailing" edges.

How does it influence the performance

Polishing reduces the possibility of fiber to lodge between the bars causing the screen to blind. The polished edges increase the "slicing" ability of the bars, thereby increasing the capacity of the screen. The polished surface is easier to clean. The special shape of the full bar enables the screen to be reversed without diminishing its effectiveness.

Benefits

Higher capacity
Less fiber in the underflow
Higher capture rate
Dryer discharge
Easier cleaning
Longer life

there's a lot behind the DSM

The DSM Screen is a unique device and is the only screen of its type which employs a continuously curved screen surface. The design of this unit and its function has been developed over many years. As a result of this developmental work, Dorr-Oliver is capable of supplying the technical backup required for sizing and proper function of the screen.

A large number of complete screens are carried in stock in the factory in various sizes, types and materials of construction. In most cases, quick delivery of a DSM can be made whether the requirement is for coarse or fine screening applications.

If it is desirable to have a demonstration of the DSM Screen for purposes of evaluating your particular process, arrangements can be made for a test (rental) unit through one of D-O's local offices.

Dorr-Oliver has acquired valuable know-how and backup information through years of extensive testing and development work; countless demonstrations on which the DSM Screen has been used; and numerous successful installations working effectively throughout the world. *That's why Dorr-Oliver is the company to help you with your screening problem.*

Various size screens are shown assembled and stored at D-O's Hazleton, Pa., factory.

Dorr-Oliver's Springdale Test Facility in Stamford, Ct., provides precise data for evaluating performance parameters of DSM Screens and other equipment.

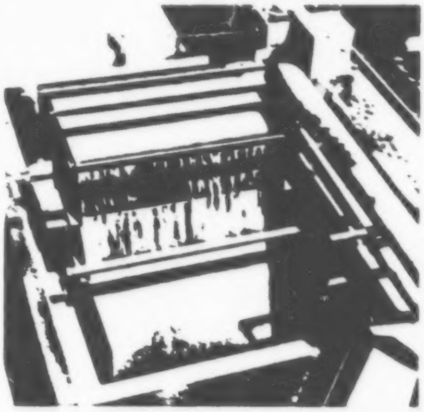
the DSM
is used in many food processes

1. Citrus

Coarse Fiber is economically removed on DSM Screens due to their high capacity/ft and low maintenance.

Example: **Citrus juice**
Equipment: **Model 6′ 45—1600**
Bar spacing: **0.7 mm**
Capacity: **110 gpm/ft**

1



2. Canning

Dewatering of vegetable waste is performed effectively and efficiently on DSM Screens.

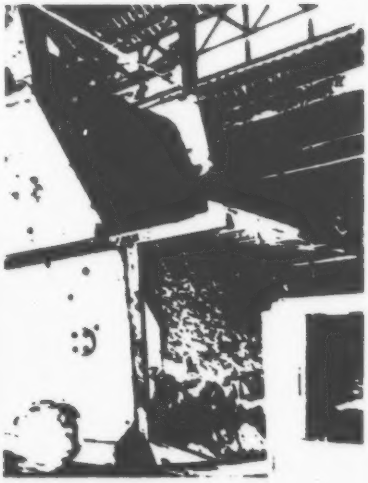
Example: **Spinach waste**
Equipment: **Model 6′ 45—1600**
Bar spacing: **2.3 mm**
Capacity: **200 gpm/ft**

3. Sugar Cane

Juice Recovery with DSM Screens is an integral part of the cane sugar flow sheet.

Example: **Primary sugar juice**
Equipment: **Model 6′ 45—1600**
Bar spacing: **1.0 mm**
Capacity: **120 gpm/ft**

3

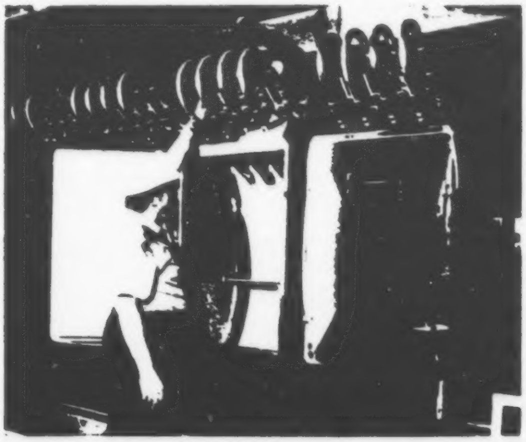


4. Corn Starch

Fine Fiber Removal using DSM Screens in a countercurrent washing system is standard practice in the corn wet milling industry.

Example: **Fine corn fiber**
Equipment: **Model 2′ 120**
Bar spacing: **0.075 mm**
Capacity: **120 gpm/ft**

4



conversion table

U.S. Standard Mesh Sizes

		U.S. MESH NUMBER	SIEVE OPENING		
			MICRONS	INCHES	MM
SEPARATION RANGE FOR	GRAVITY FED	8	2380	.0937	2.38
		10	2000	.0787	2.0
		12	1680	.0661	1.68
		14	1410	.0555	1.41
		16	1190	.0469	1.19
		18	1000	.0394	1.00
		20	840	.0331	.84
		25	710	.0280	.71
		35	500	.0197	.50
		45	350	.0138	.35
	PRESSURE FED	50	297	.0117	.297
		70	210	.0083	.210
		100	149	.0059	.149
		140	105	.0041	.105
		200	74	.0029	.074
		270	53	.0021	.053

For more information contact your nearest Dorr-Oliver regional sales office

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Tel. 312-887-7300

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Tel. 404-394-6200

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Tel. 203-358-3600

DORR-OLIVER

DORR-OLIVER INCORPORATED
77 HAVEMEYER LANE, STAMFORD, CONN. 06904

BULLETIN DSM 5
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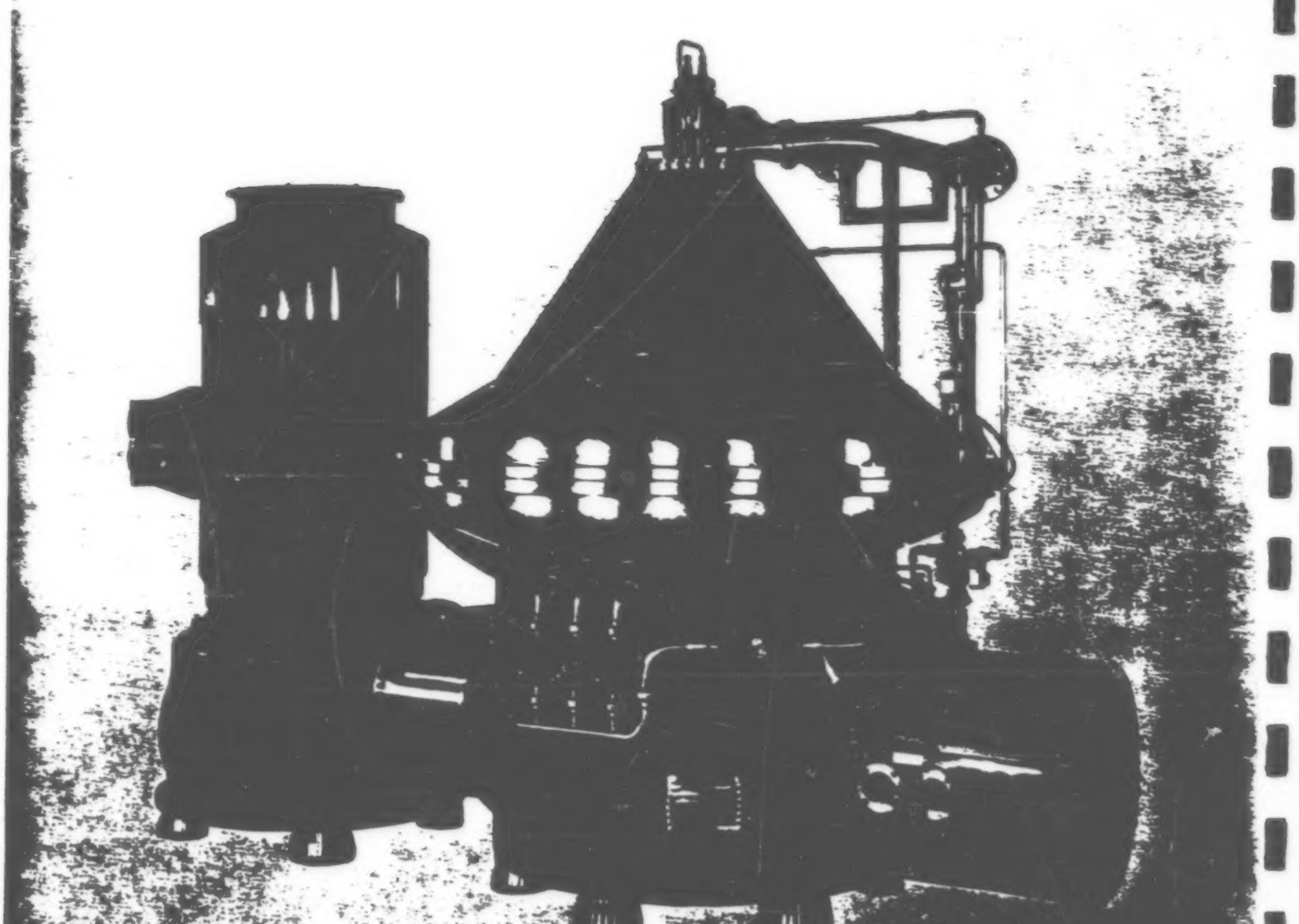
APPENDIX VII

WESTFALIA CENTRIFUGES

SPECIFICATIONS

De-Sludger/Clarifiers

Models ▶	**SA 160-06-117 SA 160-36-**
Maximum Capacity (l/h*)	150 000
Bowl Volume (liters)	125
Bowl Sludge Capacity (liters)	57
Maximum Bowl Speed (rpm*)	3 300
Motor Rating (kW)	70
Dimensions, L x W x H (mm)	2685 x 1735 x [illegible]
Gross Shipping Weight (kg)	6 500



DATA FOR DE-SLUDGERS WITH CLARIFIER BOWL

SA 100-06-777 SA 100-36-777	SB 80-06-076 SB 80-36-076	SB 80-06-177 SB 80-36-177	SA 45-06-076 SA 45-36-076	SA 45-06-177 SA 45-36-177	SA 35-06-076 SA 35-36-076	SA 20-06-076 —	SB 14-06-076 SB 14-36-076	SB 7-06-076 SB 7-36-076
55 000	35 000	35 000	35 000	35 000	22 000	12 000	8 000	4 500
46	30	30	26.5	26.5	21	12.5	6.9	3
25	17	17	16	16	11	6.5	3.9	1.5
4 870	5 000	5 000	5 000	5 000	6 500	6 500	7 500	9 200
37–45	22–30	30	22–30	25–30	18.5	11	7.5	4
1950 × 1700 × 1890	1500 × 1300 × 2200	1500 × 1300 × 2200	1500 × 1300 × 2200	1500 × 1300 × 2200	1320 × 900 × 1770	1650 × 980 × 1700	1345 × 850 × 1335	1140 × 790 × 950
3 140	2 260	2 455	2 190	2 375	1 525	1 000	1 000	336

* Actual capacity and bowl speed depend on characteristics of feed materials. (Conversion factors: 1 liter = 0.264 U.S. gallon (0.22 Imp. gallon), 1 mm = 0.039 inch; 1 kg = 2.2 lb; 1 kW = 1.34 hp)

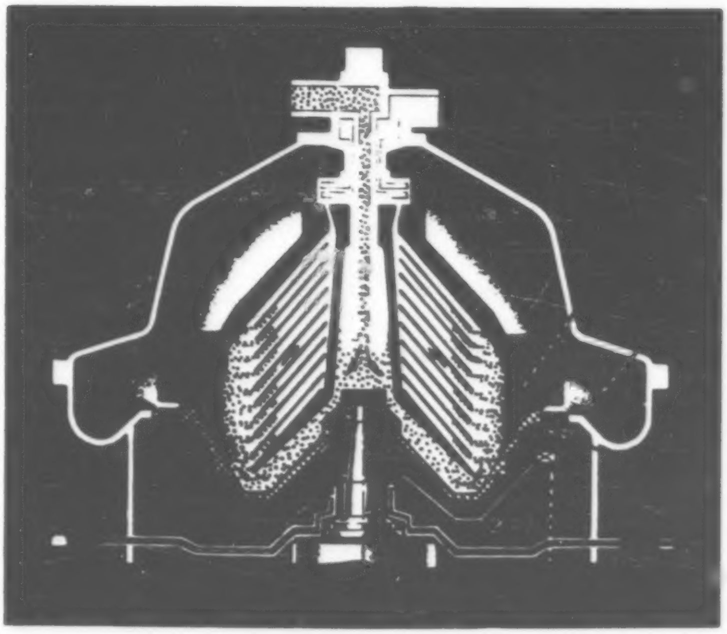
** Starting power 125 kW.

Suffix Designations

06: Standard Model; 36: "Self Thinker" Model;
076: Gear Type Motor; 177: V-Belt Drive Motor;
777: As 177 but in "low noise" design.

Automatic de-sludgers with clarifier bowl are ideal for processing of liquids containing up to 20 % solids. They are useful in the recovery or removal of solids, or in the clarification of liquids, with least liquid lost in the discharged sludge. Most de-sludgers in this category incorporate the "Liquid-SEAL" design (described on page 5); in these models clarified liquid is discharged enclosed and under pressure.

CLARIFIER BOWL



Hermetic de-sludger/clarifiers are described on page 14.

All automatic de-sludger/clarifiers offer these advantages to the user:

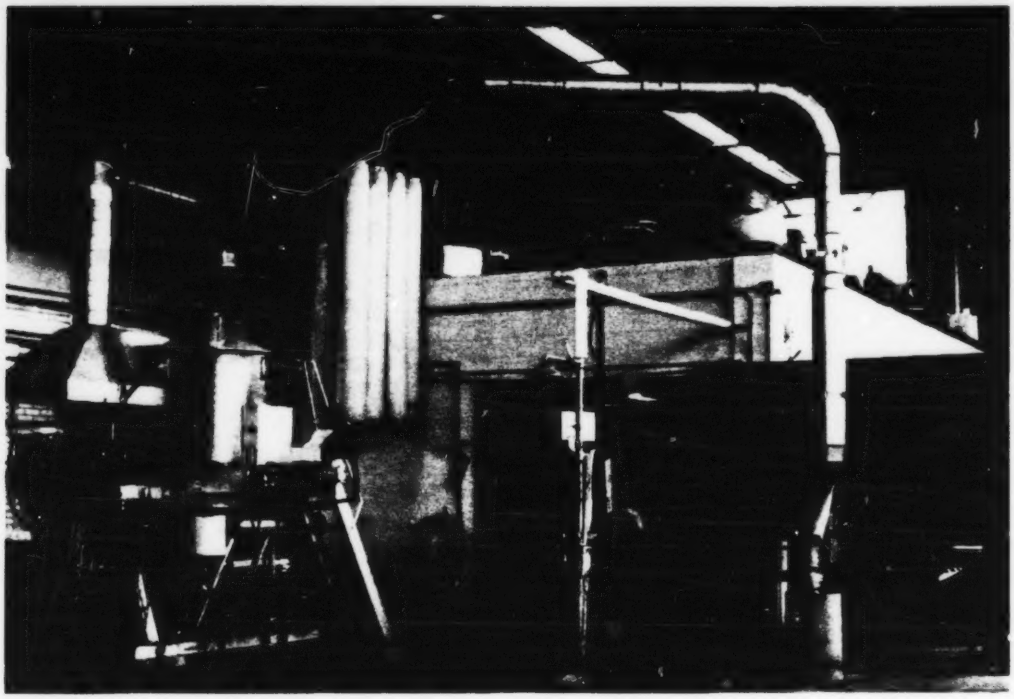
- Small Space Requirements.
- Sturdy Construction.
- Clean-In-Place Feature.
- Fully Automatic Operation.
- Simple Bowl Design for Optimum Performance.

Typical Applications

"Liquid-SEAL" de-sludger/clarifiers are used in the clarification or recovery of a wide range of chemical, food and pharmaceutical products, such as: algin liquor; atomic wastes; beer; caustic solutions; citrus, fruit and vegetable juices; coffee and tea extracts; fermentation broths; grape and berry juices; molasses; pineapple juice; protein extracts; syrups; wine.

APPENDIX VIII

PHOTOGRAPHS OF PILOT PLANT EQUIPMENT



WENGER EXTRUDER (LEFT) AND FEED SYSTEM

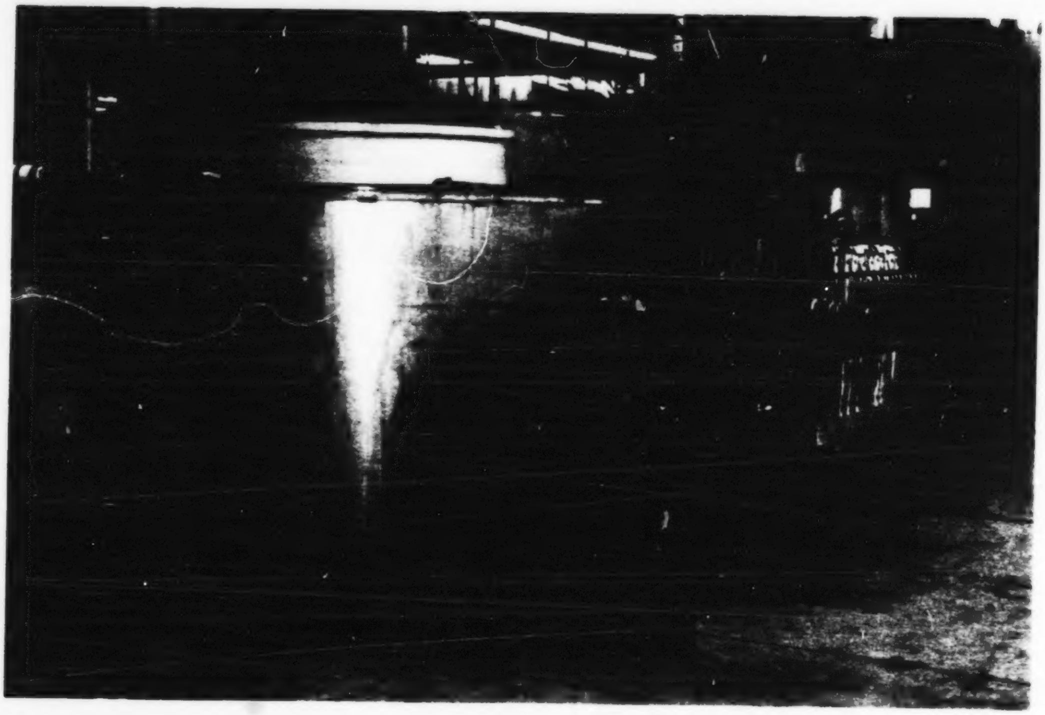
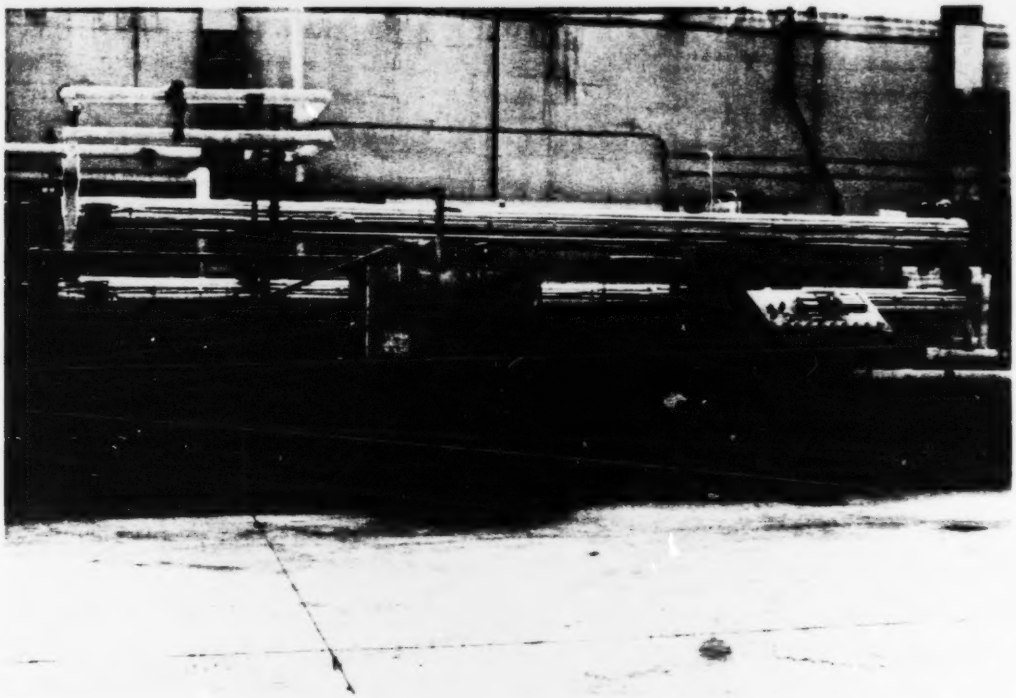
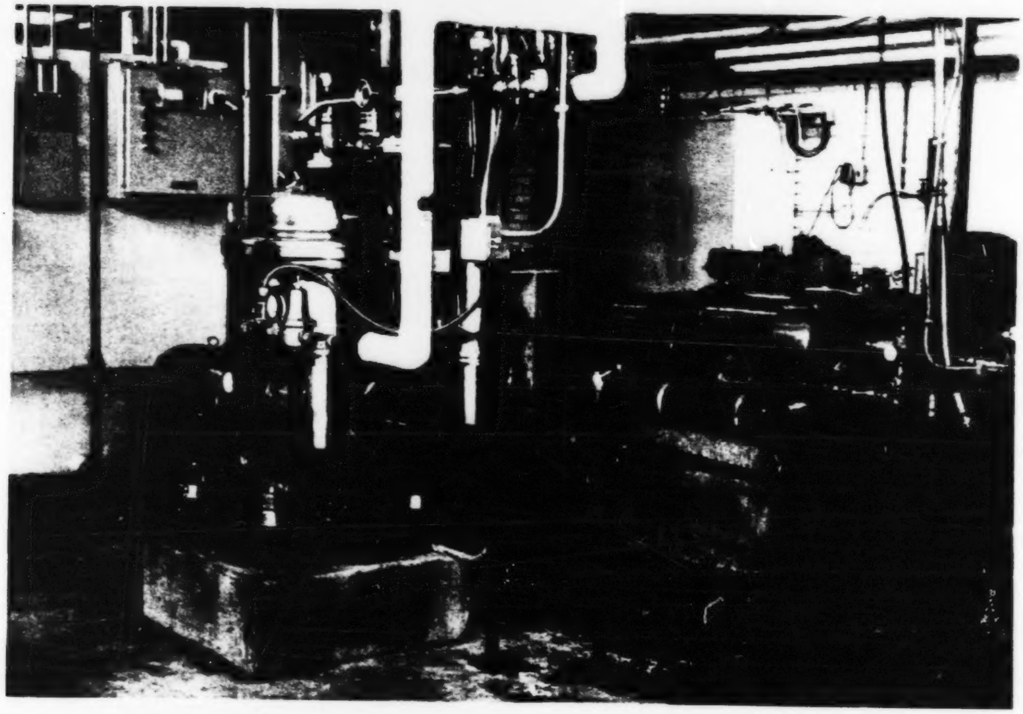


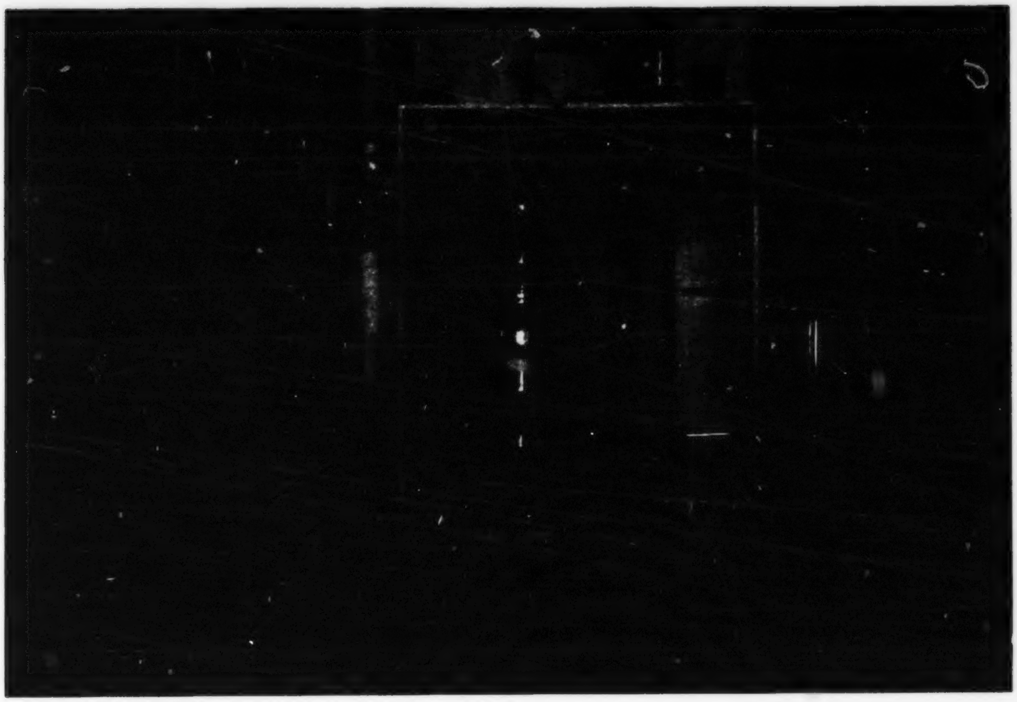
PLATE AND FRAME FILTER PRESS



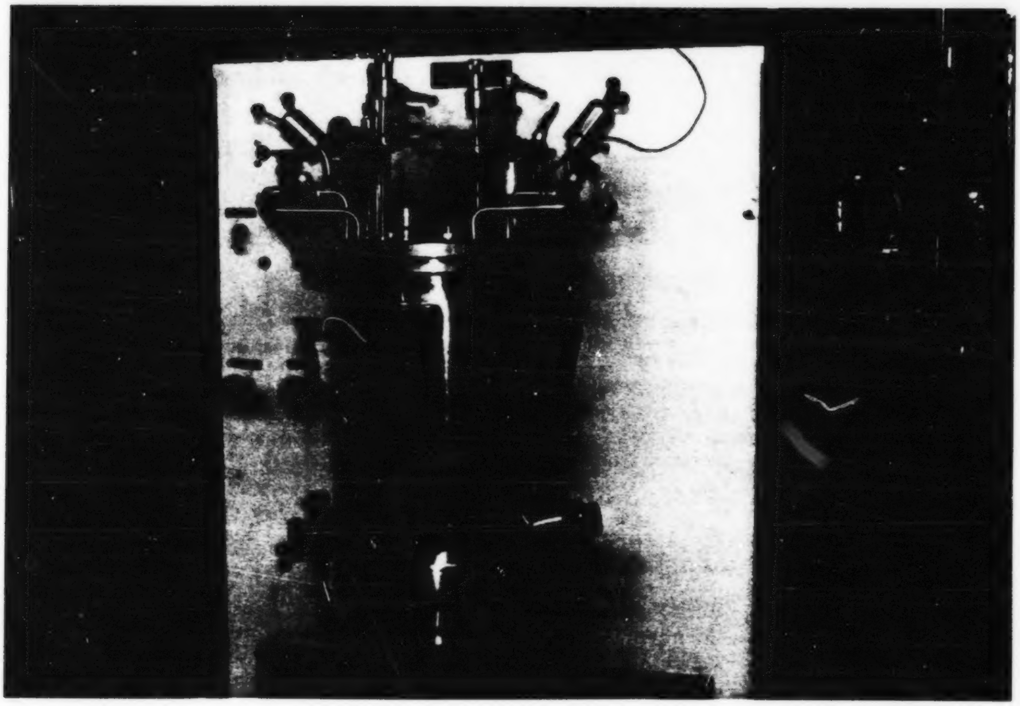
SLR PLUG FLOW REACTOR



CENTRIFUGE (LEFT) AND DECANTER



STEAM STRIPPER



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